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TECHNICAL REPORT

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Outcome of a public consultation on the draft risk assessment of perfluoroalkyl substances in food

European Food Safety Authority (EFSA)

Abstract

The European Food Safety Authority (EFSA) carried out a public consultation to receive input from interested parties on a draft scientific opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. This draft scientific opinion was prepared by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel), supported by the Working Group on Perfluoroalklyated substances in food. The draft opinion was endorsed by the CONTAM Panel for public consultation on 30 January 2020. The written public consultation was open from 24 February until 20 April 2020. EFSA received comments from 25 different interested parties. EFSA and its CONTAM Panel wish to thank all stakeholders for their contributions. The present report contains the comments received and explains the way they have been considered for finalisation of the Opinion. The Opinion was adopted at the CONTAM Plenary meeting on 9 July 2020 and published in the EFSA Journal.

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Key words: PFAS, food, exposure, mixtures, immune system, PBPK, risk assessment, public consultation

Requestor: European Commission

Question number: EFSA-Q-2019-00162

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Following the Scientific Opinion on Perfluorooctane sulfonate (PFOS) perfluorooctanoic acid (PFOA) and their salts[1], the European Commission recommended an EU-wide monitoring[2] of perfluoroalkylated substances in food. The occurrence data generated by this monitoring have been used in the Scientific Report entitled 'perfluoroalkylated substances in food: occurrence and dietary exposure'[3].

1.1.2. Terms of reference

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to prepare an opinion on the risks to human health related to the presence of perfluoroalkylated substances in food, considering existing hazard assessments and available occurrence data.

1.2. Rationale for the public consultation and brief summary of its outcome

In line with EFSA's policy on openness and transparency, and in order for EFSA to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key issues. Accordingly, the draft opinion together with its annex was released for public consultation from 24 February 2020 to 20 April 2020 by means of an electronical comment submission tool together with explanatory text on the EFSA website (See Appendix A). Comments were received from 25 interested parties from 11 countries. Table 1 provides an overview on the interested parties that have submitted comments through the electronic submission or via email.

Table 1: Overview on stakeholder comments received

Stakeholder	Category [(a)]	Country
Anthony Tweedale	Personal capacity	BE
Philippe Grandjean & Esben Budtz-Jørgensen	Personal capacity	DK
Federal Food Safety and Veterinary Office	National authority	CH
Carlotta Bargossa	Personal capacity	IT
Linda Birnbaum	Personal capacity	US
Center for Research on Ingredient Safety Michigan State University	University/public research institute	US
German Environment Agency	National authority	DE
HBM4EU, the European Human Biomonitoring Initiative	Other	AT

[1] Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluooctane sulfonate (PFOS) perfluorooctanoic acid (PFOA) and their salts. The EFSA Journal (2008) Journal number, 653, 1-131.
[2] Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food. OJ L 68, 18.3.2010, p.22.
[3] European Food Safety Authority, perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012, 10(6) 2743

Fidra	NGO	UK
Food, Nutrition and Health Unit – Istituto Superiore di Sanità	National authority	IT
Gezinsbond (FlemishFamily Organisation)	Other	BE
Rolf Soer	Personal capacity	NL
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	National authority	UK
Swedish Environmental Protection Agency	National authority	SE
Norwegian Scientific Committee for Food and Environment	National authority	NO
Daikin Chemical Europe GmbH	Private sector (e.g. industry, consultancy, etc.)	DE
FoodDrinkEurope	Other	BE
Karolinska Institutet / Institute of Environmental Medicine	University/public research institute	SE
German Federal Institute for Risk Assessment (BfR)	National authority	DE
American Chemistry Council	Private sector (e.g. industry, consultancy, etc.)	US
Ministerium für Ländlichen Raum und Verbraucherschutz Baden Württemberg	National authority	DE
National Institute for Public Health and the Environment (RIVM)	University/public research institute	NL
The 3M Company	Private sector (e.g. industry, consultancy, etc.)	US
Mamme No Pfas	Other	IT
Federation of the European Cutlery, flatware, hollowware and cookware industries (FEC)	Not specified	FR

(a): As specified by the commenter.

2. Assessment of comments and use for finalisation of the opinion

The comments received were duly evaluated by the EFSA WG on Perfluoroalkylated substances in food and the CONTAM Panel, and wherever appropriate, taken into account in the finalisation of the draft opinion. Table 2 provides a detailed list with all comments as received from interested parties together with EFSA responses and explanations how the comments were considered for finalisation of the draft opinion. Translations from Italian to English were provided to EFSA by the Centre de Translations, CdT, in Luxembourg, for comment numbers 204-208; 213-215.

Table 2: Stakeholder comments and EFSA responses

Stakeholder	Comment number	Chapter	Comment	EFSA response
Anthony Tweedale	1	2. Data and Methodology	During CONTAM Panel's public session (online) on 12 March 2020, the response to one of my two questions invited me to submit the PFAS toxicity studies that I had said might not have been evaluated ...but I don't know which member invited me to submit the information to her! Anyway, PubMed currently returns 3,587 studies for the below search term. Adding Web of Science (WoS) is a typical search strategy for published findings, but I don't have access to it. Assuming 20% of PubMed's findings would be indexed only in WoS journals, one might find 4,300 *potential* toxicity papers. In my experience 50% and 80% turn out to be toxicity findings (vivo, vitro or epidemiology). So, ~2,150 to 3,440 published PFAS toxicity findings may exist today. CONTAM cited a few hundred in its 900 items reference list, and no doubt evaluated more than that--but how many more? If you did not reach 2,000 that screen as initially relevant (i.e. seem to be toxicity findings); I would try more search terms. -- ("PFOA" OR "PFOS" OR "PFAS" OR "perfluoro*" OR "polyfluoro*") AND (effect* OR hazard* OR risk* OR toxic* OR safet*)	There may be several reasons why the numbers retrieved by a literature search and those in the reference list differ. First of all, the CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Secondly, literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N). The paper by Abraham et al. (2020) (Archives of Toxicology, 94:2131-2147), which the CONTAM Panel was made aware of, confirmed the results from other papers and contained useful dose-response information for the assessment. In addition, the authors provided information on the sum of the 4 PFASs, upon request from the CONTAM Panel. It was clear that the paper would be published in time for the public consultation. Furthermore, all relevant information was included in the opinion.
Anthony Tweedale	2	4. Conclusions	During the CONTAM public comment meeting, I incorrectly wrote in a comment that the NTP's new PFOA study tested 20 pptrillion. In fact, this lowest dose tested was 20 ppm (I had been looking that morning at a study on trichlorethene which did use 20 pptrillion in drinking water, and probably conflated the two in my mind). No doubt EFSA & CONTAM will have figured out the truth!	Noted.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Anthony Tweedale	3	4. Conclusions	May I add that the NTP PFOS study also found clear elevations in mammary gland problems, including lactation--a confirmation of PFOA's 3 times lower (than your immune TWI) breast gland toxicity finding--which you discounted, saying you will keep on eye on it. Is this independent confirmation convince you to switch key studies, for a lower TWI (safe dose)?	To our knowledge, the recent PFOS assessment by NTP (2019b) did not investigate effects on mammary gland development after perinatal exposure so it is not clear to which study the stakeholder is referring. Regarding effects on lactation, the only study which was identified investigating this was the three-generation study by White et al., 2011 (described in the Opinion Section 3.3.3.3.2). There is a recommendation to study effects of other PFASs on mammary gland development.
Anthony Tweedale	4	Appendix N	All my browsers recognised, but none would actually load, Bull et al. '14--where CONTAM describes the literature search strategy. But I did see the search term used for toxicity, in Appendix N.	Bull et al. (2014) can be found here: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2014.EN-572
Philippe Grandjean & Esben Budtz-Jørgensen	5	-	This contribution has been generated jointly with Esben Budtz-Jørgensen, email: ebj@sund.ku.dk. Both names should appear as authors. The specific biostatistical comments are uploaded as a separate file, as the pdf format allows better presentation of the equations and tables that are includes in the form below.	Thank you, this has been noted.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	6	Abstract	We much appreciate the opportunity to comment on the new draft opinion on PFASs that was made available for public comments. Our below comments are not meant as a thorough review of the full draft opinion, as we have focused on the validity of assessing joint exposures to four PFASs, the evaluation of human health effects, especially the immunotoxic effects, and the approach to developing tolerable intake levels. We note that we were both part of the authorship that generated the EFSA guidelines for using Benchmark Dose (BMD) calculations (1), and we have published several articles on PFAS immunotoxicity in recent years, some of which are cited below. 1. EFSA Scientific Committee (EFSA). Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. The EFSA Journal. 2009;1150:1-72.	The present Opinion was based on the most recent BMD guidance*. *EFSA Scientific Committee, 2017. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. doi:10.2903/j.efsa. 2017.4658
Philippe Grandjean & Esben Budtz-Jørgensen	7	Summary	Given that the four PFASs may not be equivalent in their toxicological potencies, the summation of their concentrations in blood serum may result in increased imprecision of the exposure assessment. In our prospective study of a Faroese birth cohort, for example, the relative contribution by each of the four PFASs to the total PFAS concentration (on a weight basis) varied substantially in the 545 children, whose serum was analysed at age 5 years (2): Min Max Mean Std PFOS 0,44 0,88 0,74 0,062 PFOA 0,08 0,35 0,18 0,048 PFHxS 0,00 0,40 0,03 0,024 PFNA 0,02 0,16 0,05 0,019 This means that, PFOS contributed between 44% and 88% of the total amount of PFAS in serum from these children, while PFHxS contributed between 0% and 40% (EFSA believes that PFHxS and PFNA contribute little to the overall exposure, p. 163, line 6134). These variations in the PFAS constitution could be of importance, depending on the	The CONTAM Panel agrees that relative potencies for immune effects are lacking and that these, if they had been available, should ideally have been derived on a molar base. In the absence of such factors, The CONTAM Panel considered it more appropriate to sum the PFASs for which sufficient information was available, rather than to ascribe the association to just one of the PFASs, derive a critical serum level, and then repeat this for the next PFAS. For those PFASs not substantially contributing to the serum levels, it implies that they are not included in the HBGV, whereas structural similarities and persistence in humans warrant a mixture approach. Based on a comparison of the 2018 Opinion and the new one, it also appears that the association observed for the data from the Faroese islands is better for the sum than for PFOS. Considering the use of molar versus weight based relative potencies, it should be noticed that it is common practice to round these values e.g. on a log scale (see dioxins and dioxin-like PCBs). Furthermore, for chemical analysis it is more pragmatic to apply weight-based relative potencies. Therefore, it was decided to base the assumption of equal potencies on a weight base, assuming that the overall

Stakeholder	Comment number	Chapter	Comment	EFSA response
			differences in the relative potency of the four PFASs. Our findings suggest that using the arithmetic sum of the four concentrations may generate mixture concentrations that are uneven, and any exposure-related effects will therefore depend on both the different potencies of the PFASs and on their relative concentrations. Their molecular weights also differ: Mol w (%PFOS) PFOS 500* 100 PFOA 414 83 PFHxS 400 80 PFNA 464 93 *EFSA uses a molecular weight of 538 for PFOS (p. 162, line 6124) This means that the same mass of PFAS will contain 20% more PFOA molecules, 25% more PFHxS, and 8% more PFNA than PFOS, i.e., on a molar basis. If equimolar potency is assumed, that would require using the molar concentrations that would result in a greater relative contribution from PFOS. This issue adds an additional element of uncertainty to the calculations carried out by EFSA. However, for the sake of easier comparison, EFSA ignores the molar weights and states that the differences contribute "little" to the uncertainty (p. 162, lines 6121-5). 2. Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 2012;307:391-7.	uncertainties in the assessment would outweigh the differences introduced by using weight-based potencies.
Philippe Grandjean & Esben Budtz-Jørgensen	8	1. Introduction	No further comments.	Noted

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	9	2. Data and Methodology	Because laboratory animal studies may not be predictive for human PFAS toxicity, EFSA appropriately focuses on human studies. However, it appears that EFSA considers studies without significant associations between exposure and outcome as indication that a causal link does not exist. Still, some studies may have been too small to allow for informative statistical power, some used either concurrent or recent exposure data where perhaps developmental data would have been more appropriate, and some studies had other weaknesses. It seems to us that EFSA did not consider the weight of the evidence when listing the studies considered. In a precautionary context, potential biases towards the null need attention (3). We list below some concerns in regard to several organ systems and outcomes, where we believe that EFSA's assessment lacks detail and proper assessment of the strength of the evidence. Notwithstanding these concerns, we agree with the selection of the immune system as the most likely critical organ, for which protective exposure limits should be calculated. 3. Grandjean P. Science for precautionary decision-making. In: Gee D, Grandjean, P., Hansen, S.F., van den Hove, S., MacGarvin, M., Martin, J., Nielsen, G., Quist, D., Stanners, D., editors. Late Lessons from Early Warnings. II. Copenhagen: European Environment Agency; 2013. p. 517-35.	The risk assessment is based on both animal and human studies. The Opinion aims to strike a reasonable balance between focus on significance and weight of evidence. Several of the human studies on antibody response showed a nonsignificant trend that supported the overall evidence for that outcome.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	10	2.1.3 Toxicokinetic and Toxicological data	As the adverse effects are seen in children, the corresponding maternal intake must be assessed, as EFSA has done. Thus, EFSA uses a transgenerational calculation that has similarities to the model used by the state of Minnesota (4). We support this approach, given the very substantial developmental exposure that occurs through placental passage and also through excretion into human milk (5). However, we note that there is some uncertainty involved, as transplacental transfer is increased in diabetic women (6), and because some data on PFHxS suggests a lower transfer into human milk than for, e.g., PFOS (5). 4. Goeden HM, Greene CW, Jacobus JA. A transgenerational toxicokinetic model and its use in derivation of Minnesota PFOA water guidance. J Expo Sci Environ Epidemiol. 2019;29:183-95. 5. Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jorgensen E. Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates. Environ Sci Technol. 2015;49:10466-73. 6. Eryasa B, Grandjean P, Nielsen F, Valvi D, Zmirou-Navier D, Sunderland E, Weihe P, Oulhote Y. Physico-chemical properties and gestational diabetes predict transplacental transfer and partitioning of perfluoroalkyl substances. Environ Int. 2019;130:104874.	Model evaluation (uncertainty, sensitivity) has now been added to Appendix M according to the WHO/IPCS (2010) guidance. The notion of uncertainty encompasses both true uncertainty (i.e. in model parameter value) and variability (i.e. from population variability). The CONTAM Panel performed a sensitivity and uncertainty analysis for placental transfer, for the latter, varying between 0.37 and 1.11 (with a median of 0.74) for PFOA, and 0.18 and 0.54 (with a median of 0.36) for PFOS. It appears that such variations have a minor influence on the serum concentration estimated at 1 year of age with the PBPK model. According to the WHO/IPCS guidance, the placental transfer value chosen by the CONTAM Panel appears to have a high reliability.
Philippe Grandjean & Esben Budtz-Jørgensen	11	2.2. Methodologies	Our comments are in the specific subsections.	Noted.
Philippe Grandjean & Esben Budtz-Jørgensen	12	2.2.2 Literature search and appraisal of studies	It seems that EFSA's literature search captured articles published as late as in 2019 and thus should be reasonably up to date. However, we note that the literature coverage is uneven, and this is particularly concerning when large prospective studies are left out. We have not carried out a systematic comparison, but EFSA missed a large prospective study of diabetes (7) and an intervention study of body	The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a

Stakeholder	Comment number	Chapter	Comment	EFSA response
			weight (8), both published in major journals in 2018. We must suspect that EFSA's dismissal of metabolic disease as a potential outcome of PFAS exposure may have to do with the incomplete literature coverage coupled with the tendency of counting studies rather than assessing the weight of the epidemiological evidence. 7. Sun Q, Zong G, Valvi D, Nielsen F, Coull B, Grandjean P. Plasma Concentrations of Perfluoroalkyl Substances and Risk of Type 2 Diabetes: A Prospective Investigation among U.S. Women. Environ Health Perspect. 2018;126:037001. 8. Liu G, Dhana K, Furtado JD, Rood J, Zong G, Liang L, et al. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Med. 2018;15:e1002502.	draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N).
Philippe Grandjean & Esben Budtz-Jørgensen	13	2.2.3 Methodology applied for risk assessment	We note that EFSA has previously provided detailed guidance on calculating the Benchmark Dose and its lower 95% confidence limit, the Benchmark Dose Level (BMDL), for the purpose of identifying tolerable intake levels (1). In this connection, EFSA argued against the use of NOAEL, as this approach is associated with much uncertainty and does not utilize the full information available from the data. It is therefore surprising to us that EFSA in the present draft opinion reverts to using the NOAEL approach for epidemiology data (p. 154, line 5833 ff). As justification, EFSA refers to the wide confidence intervals around BMD results, although using the lower confidence limit would seem to be in accordance with the precautionary principle (9). Also, EFSA refers to the absence of an unexposed control group. Again, this is a well-known issue, and it can be approached in different ways (10). The decision not to use an uncertainty factor is surprising. EFSA uses segmented regression, though without confidence intervals. A better solution to the unknown dose-response (DR) curve in the Benchmark analysis would be based on a flexible non-linear function such as, e.g., the K-power model (11).	The current guidance on BMD modelling* clearly states that existing guidance was developed around modelling of experimental studies in animals. Although the same methodology can in principle be applied to human studies, no guidance currently exists. In its recommendation, the Scientific Committee '*identifies the need for a specific guidance on the use of the BMD approach to analyse human data*'. In the absence of guidance on how to model human data taking into consideration the uncertainties around having no zero dose and a wide confidence interval that partly depends on sample size, it was initially decided to go for the NOAEC approach. However, as a response to requests from several stakeholders (including you), this was reconsidered and in the revised opinion the BMD approach is used to derive a reference point. *EFSA Scientific Committee, 2017. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. doi:10.2903/j.efsa. 2017.4658

Stakeholder	Comment number	Chapter	Comment	EFSA response
			9. Budtz-Jorgensen E, Keiding N, Grandjean P. Benchmark dose calculation from epidemiological data. Biometrics. 2001;57:698-706. 10. Grandjean P, Budtz-Jorgensen E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environ Health. 2013;12:35. 11. Crump KS. Calculation of benchmark doses from continuous data. Risk Analysis. 1995;15:79-89.	
Philippe Grandjean & Esben Budtz-Jørgensen	14	3. Assessment	We provide comments below in regard to the specific concerns.	Noted.
Philippe Grandjean & Esben Budtz-Jørgensen	15	3.3.1.2 Humans (Toxicokinetic)	Notwithstanding our below reservations, we agree on the selection of the immune system as the most likely critical organ, for which protective exposure limits should be calculated. We note that IARC mentioned immunotoxicity as a possible key characteristic that may lead to cancer development (12). In the draft opinion, EFSA relies on unpublished evidence, i.e., a study by Klaus Abraham and colleagues. We contacted the EFSA representatives, with whom we had shared our own data, and requested a copy of the Abraham et al. publication but received no response. We therefore contacted Dr. Abraham ourselves to obtain a copy of the manuscript. That allowed us to carry out calculations using the results from the German study that EFSA had relied on. 12. International Agency for Research on Cancer. Some Chemicals Used as Solvents and in Polymer Manufacture. Perfluorooctanoic acid. 2016. In: IARC Monographs On The Evaluation Of Carcinogenic Risks To Humans. Lyon: International Agency for Research on Cancer; pp. 37-110.	EFSA is strongly supportive of sharing all data, both for human studies and animal studies. It is not uncommon for the CONTAM Panel to use non-published data for risk assessments, but it is evident that the essential information should be available. In this case, due to data protection, the CONTAM Panel was unable to obtain individual data but, on request, additional aggregated data were obtained on the sum of the 4 PFASs and these were described in the draft Opinion. The paper by Abraham et al. (2020), which focuses on PFOA, is published.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	16	3.3.4 Observations in humans	Because laboratory animal studies may not be predictive for human PFAS toxicity, EFSA appropriately focuses on human studies. However, it appears that EFSA considers studies without significant associations between exposure and outcome as indication that a causal link does not exist. Still, some studies may have been too small to allow for informative statistical power, some used either concurrent or recent exposure data where perhaps developmental data would have been more appropriate, and some studies had other weaknesses. It seems to us that EFSA did not consider the weight of the evidence when listing the studies considered. In a precautionary context, potential biases towards the null need attention (3). See also above on important prospective studies missed by EFSA.	Please see answer to comment number 9 above.
Philippe Grandjean & Esben Budtz-Jørgensen	17	3.3.4.4 Immune outcomes	We note that EFSA refers to some of the studies on antibody concentrations as "observational" (p.122, lines 4443ff), but it is not clear what is meant, as all nonexperimental epidemiology is by nature observational. An important advantage of the vaccine antibody studies is that they are interventional and rely on a standardized antigen challenge at a well-defined age followed by routine clinical laboratory assessment of specific antibody concentrations.	The CONTAM Panel acknowledges the interventional non-experimental component of the vaccination studies and it played an important role when weighing the overall evidence.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	18	3.3.4.4.2 Vaccination response	We agree that responses to vaccinations in terms of concentrations of specific antibodies can be used as a clinically relevant marker of immune dysfunction (13). This approach has been used to characterize immunotoxic effects of, e.g., PCBs and dioxins (14-17), including our own study of PCB-exposed children (18) that served as a proof-of-principle. While EFSA cites most of the relevant information in this regard, we wish to add some additional aspects that we believe may strengthen the conclusion. We also call attention to the excellent review by DeWitt that provides a useful perspective (19). In our own prospective study in the Faroes (2, 20), we noted that a substantial number of highly exposed children at age 7 had antibody concentrations that were so low that they had no long-term protection against the targeted diseases despite a total of four vaccinations. Our findings are consistent with a small Norwegian study (21) that linked elevated PFAS exposures also to increase occurrence of common cold and gastroenteritis. In our replication study, we found that the estimated exposure at age 3 months was a better predictor of age-5-year antibody concentrations than was the serum concentration at age 5 (22). 13. Heilmann C, Grandjean P. Immunotoxicity: Impacts and Research Approaches. In: Kishi R, Grandjean P, editors. Health Impacts of Developmental Exposure to Environmental Chemicals. Singapore: Springer; 2020. pp. 175-90. 14. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect. 2000;108:1203-7. 15. Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jorgensen E. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med. 2006;3:e311. 16. Stolevik SB, Nygaard UC, Namork E, Haugen M, Meltzer HM, Alexander J, et al. Prenatal exposure to polychlorinated	Thank you for your comment. Based on PBPK modelling, it appears that the serum levels at 3 months are primarily determined by exposure via the mother, whereas at 5 years it is determined by regular food intake. If the response at 7 years was determined by perinatal exposure, this would suggest a long lasting effect of PFASs on the immune response. The issue is, could this also explain the association between serum PFAS levels at 5 years of age and antibody titres at 7 years? It would be interesting to apply the PBPK model on the data of the Faroe islands to obtain further insight. The Granum et al. (2013) study is described in the Opinion but was considered less suitable for deriving the Reference Point. Nevertheless, EFSA obtained the individual data and performed a BMD analysis, showing a dose-response for the association between serum levels of the sum of 4 PFASs and antibody titres against rubella. This information has been added to Appendix K.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood. Food Chem Toxicol. 2013;51:165-72. 17. Jusko TA, De Roos AJ, Lee SY, Thevenet-Morrison K, Schwartz SM, Verner MA, et al. A Birth Cohort Study of Maternal and Infant Serum PCB-153 and DDE Concentrations and Responses to Infant Tuberculosis Vaccination. Environ Health Perspect. 2016;124:813-21. 18. Heilmann C, Budtz-Jorgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect. 2010;118:1434-8. 19. DeWitt JC, Blossom SJ, Schaider LA. Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence. J Expo Sci Environ Epidemiol. 2019;29:148-56. 20. Mogensen UB, Budtz-Jørgensen E, Heilmann C, Nielsen F, Weihe P, Grandjean P. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated compounds. Environ Health. 2015;49:10466-73. 21. Granum B, Haug LS, Namork E, Stolevik SB, Thomsen C, Aaberge IS, et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol. 2013;10:373-9. 22. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A, et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol. 2017;14:188-95.	

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	19	3.3.4.4.3 Clinical Infections	An extension of the Norwegian study also linked the PFAS exposure to the frequency of respiratory tract infections (23). In parallel, a study of 359 Danish children from the Odense Child Cohort found that similarly elevated maternal serum concentrations of PFOA and PFOS in early pregnancy was significantly associated with a higher frequency of fever and symptoms in their children (24). The children were monitored at ages 1-3 years for the occurrence of fever and associated symptoms every 2 weeks for a year (via text messages). The number of days with fever >38.5ºC, also in combination with nasal discharge or cough, was significantly increased in association with elevated maternal serum concentrations of PFOA and PFOS (24). Findings from Japan showed a similar tendency in 4-years old children of the general population (25), where significant trends were seen for both PFOS and PFHxS at similar levels. On the other hand, the C8 Panel concluded that there was no evidence of a link between PFOA exposure and infections (26). However, this study relied upon self-reported cold and influenza episodes, where only 71.3% of the adults interviewed reported at least a single cold during the past 12 months. Given that adults, according to the U.S. Centers for Disease Control and Prevention (https://www.cdc.gov/dotw/common-cold/index.html), have an average of 2-3 colds per year, the self-reported information most likely underreported the true occurrence of infectious disease. Thus, this study cannot support a conclusion that elevated PFAS exposure is not associated with infectious disease. As part of the studies of the Danish National Birth Cohort, early pregnancy serum from randomly selected 1400 women and their offspring were analysed by 3M for PFOS and PFOA (27). Hospitalizations for infection of the offspring were identified by linkage to the National Hospital Discharge Register, through to age 11 years. Diagnoses, such as airway infection, appendicitis, and middle ear infection, were merged, and no clear pattern was observed, also when results were stratified by child's age at infection. In addition to relying only on exposures during early gestation,	In the summary of the section (last paragraph) it is stated that '*Based on the results from the vaccination studies reviewed above, findings on increased propensity of infections would seem plausible. …… Overall, there is some evidence to suggest that exposures to PFASs are associated with increased propensity of infections but more studies with objective measures of infections (not self-reports) are needed.*' The strength of the Odense Child Cohort study is also acknowledged. Different views may exist on how to weigh the evidence on infections, but these also contributed to concluding that immune function is the most critical outcome. However, the CONTAM Panel remains of the opinion that the decrease in vaccination response should be regarded as a risk factor for disease rather than a disease.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			a recent report from the same authors raised doubt about the validity of the chemical analyses (28), thus casting doubt upon the external validity of this study. Given that the lower antibody responses to specific vaccines are associated with increased occurrence of infectious disease, we believe that these outcomes should be considered adverse effects of clinical relevance. However, EFSA considers the markers of immunotoxicity as "risk factors for disease rather than disease" (p- 158, lines 5947f), although this view is does not seem justified. We believe that substantially lowered concentrations of vaccine antibody concentrations and the associated increased incidence of infectious disease should be considered an adverse effect. The default procedures would require that EFSA applied an uncertainty factor when calculating tolerable intake levels. Because the study results reflect the average relationship between exposures and outcomes, the uncertainty factor would represent the differences in individual vulnerability. However, EFSA has chosen to leave out an uncertainty factor (p. 158, line 5945) and, in our view, does not provide proper justification. This choice is even more surprising, as EFSA relies on a NOAEL rather than a BMDL. 23. Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res. 2018;160:518-23. 24. Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Host A, et al. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4 years among 359 children in the Odense Child Cohort. Environ Int. 2016;96:58-64. 25. Goudarzi H, Miyashita C, Okada E, Kashino I, Chen CJ, Ito S, et al. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4years of age. Environ Int. 2017;104:132-8.	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			26. Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci. 2014;138:76-88. 27. Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 2010;110:773-7. 28. Bach CC, Henriksen TB, Bossi R, Bech BH, Fuglsang J, Olsen J, et al. Perfluoroalkyl Acid Concentrations in Blood Samples Subjected to Transportation and Processing Delay. PLoS One. 2015;10:e0137768.	
Philippe Grandjean & Esben Budtz-Jørgensen	20	3.3.4.6.1 Blood lipids	Most studies have focused on total cholesterol, and/or high- and low-density fractions. A recent study examined the subfractions bound to particular lipoproteins and identified the apoC-III subfraction as the one that shows the strongest increase at elevated PFAS exposures (29). This particular fraction is also strongly linked to an increased risk of cardiovascular disease and should therefore be regarded as an important potential outcome of elevated PFAS exposure. The consideration of cholesterol as a nonspecific outcome parameter also deserves EFSA's attention when evaluating hepatotoxicity, lipid metabolism, and the possible link to cardiovascular disease (30). 29. Liu G, Zhang B, Hu Y, Rood J, Liang L, Qi L, et al. Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environ Health. 2020;19:5. 30. Huang M, Jiao J, Zhuang P, Chen X, Wang J, Zhang Y. Serum polyfluoroalkyl chemicals are associated with risk of	The CONTAM Panel agrees that apolipoproteins are important risk factors for atherosclerosis and thereby for cardiovascular disease (for example ApoB in relation to cholesterol and ApoCIII in relation to triglycerides). Therefore, studies examining associations between PFAS exposure and specific plasma lipid fractions are welcome, for example the study by Liu et al. (2020) This study was published after the deadline of the literature review performed by EFSA, and was therefore not reviewed for the present Opinion (see Appendix N for further details). The study by Huang et al., 2018, was included in the opinion in Section 3.3.4.9 and Appendix J.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			cardiovascular diseases in national US population. Environ Int. 2018;119:37-46.	
Philippe Grandjean & Esben Budtz-Jørgensen	21	3.3.4.6.2 Diabetes, Obesity and Metabolic Syndrome	As noted above, EFSA missed a large prospective study of diabetes (7) and an intervention study of body weight (8), both published in major journals in 2018. We must suspect that EFSA's dismissal of metabolic disease as a potential outcome of PFAS exposure may have to do with the incomplete literature coverage coupled with the tendency of counting studies rather than assessing the weight of the epidemiological evidence. These outcomes are of very substantial public health relevance and therefore need to be properly assessed. 7. Sun Q, Zong G, Valvi D, Nielsen F, Coull B, Grandjean P. Plasma Concentrations of Perfluoroalkyl Substances and Risk of Type 2 Diabetes: A Prospective Investigation among U.S. Women. Environ Health Perspect. 2018;126:037001. 8. Liu G, Dhana K, Furtado JD, Rood J, Zong G, Liang L, et al. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Med. 2018;15:e1002502.	See response to comment 12.
Philippe Grandjean & Esben Budtz-Jørgensen	22	3.3.4.6.3 Liver	Liver toxicity may represent a relevant outcome, where serum-cholesterol is a main parameter.	Liver toxicity in experimental animals has been reviewed in Section 3.3.3.2 in the present opinion, and extensively in the 2018 Opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018). Liver toxicity in humans has been reviewed in Ssection 3.3.4.6.3 in the present opinion and in Section 3.3.4.7.2 in the 2018 opinion on PFOS and PFOA. Most of the studies in humans have focussed on serum levels of liver enzymes rather than the possible impact of liver toxicity on serum cholesterol. The CONTAM Panel agree, that changes in cholesterol metabolism could be linked to liver toxicity. Overall, the evidence of adverse effects on the liver in humans was considered weaker than the evidence of effects on the immune system.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	23	3.3.4.8 Carcinogenicity outcomes	In regard to cancer, we agree that little information has been published since the IARC evaluation of PFOA (12). Of interest, IARC mentioned immunotoxicity as a possible key characteristic that may lead to cancer development. Among recent reports is a mortality study from Italy (31) that showed an elevated cancer rate. In the USA, state agencies have carried out cancer incidence and mortality studies of residents exposed to elevated PFAS concentrations in drinking water, e.g., northern Kent county in Michigan and Merrimack, New Hampshire. In addition, a report on cancer in the East Metro area was generated for the Minnesota government (32). All of these studies support the hypothesis of elevated PFAS exposure being associated with an increased risk of cancer at certain sites, such as kidney, testes, prostate, bladder, and possibly other sites. As cancer is an important public-health concern, we urge EFSA to consider all reliable evidence, also the reports produced by or for governmental agencies. 31. Girardi P, Merler E. A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Environ Res. 2019;179(Pt A):108743. 32. Sunding DL. Damage to Minnesota's Natural Resources Resulting from 3M's Disposal of PFCs in Washington County, MN Minneapolis, MN: State of Minnesota District Court for the County of Hennepin Fourth Judicial District, Civil Action No. 27-cv-10-28862, State of Minnesota, et al. v. 3M company; 22 September, 2017.	Thank you for your comment. The CONTAM Panel considered all relevant peer reviewed literature, reports and assessment from other risk assessment bodies. The study by Girardi and Merler (2019), was published after the literature search inclusion dates for this endpoint. High doses in workers are poorly relevant to identify risks of the general population exposed through the diet.
Philippe Grandjean & Esben Budtz-Jørgensen	24	3.3.4.9 Cardiovascular disease and mortality	Se above under liver and lipids	Please refer to the replies given for comment numbers 20 and 22.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	25	3.3.5.1.3 Thyroid hormone levels and function (MoA)	We agree that the thyroid gland may be highly vulnerable (33), as we have also observed (34). Thyroid toxicity during pregnancy may result in serious consequences for brain development in the foetus (35), although developmental neurotoxicity of PFAS exposure has not yet been convincingly documented. 33. Ballesteros V, Costa O, Iniguez C, Fletcher T, Ballester F, Lopez-Espinosa MJ. Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: A systematic review of epidemiologic studies. Environ Int. 2017;99:15-28. 34. Xiao C, Grandjean P, Valvi D, Nielsen F, Jensen TK, Weihe P, et al. Associations of exposure to perfluoroalkyl substances with thyroid hormone concentrations and birth size. J Clin Endocrinol Metab. 2020;105:dgz147. 35. Leung AM, Korevaar TI, Peeters RP, Zoeller RT, Kohrle J, Duntas LH, et al. Exposure to Thyroid-Disrupting Chemicals: A Transatlantic Call for Action. Thyroid. 2016;26:479-80.	The CONTAM Panel notes this comment.
Philippe Grandjean & Esben Budtz-Jørgensen	26	3.3.5.2 Liver toxicity (MoA)	Liver toxicity may represent a major impact, where serum-cholesterol may be a key parameter, as discussed above under lipids. Most studies have focused on total cholesterol, and/or high- and low-density fractions. A recent study examined the subfractions bound to particular lipoproteins and identified the apoC-III subfraction as the one that shows the strongest increase at elevated PFAS exposures (29) and is at the same time an important marker of cardiovascular risk.	Please refer to reply given for comment number 20.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	27	3.3.5.4 Immunotoxicity (MoA)	Given that the lower antibody responses to specific vaccines are associated with increased occurrence of infectious disease, we believe that these outcomes should be considered adverse effects of clinical relevance. However, EFSA considers the markers of immunotoxicity as "risk factors for disease rather than disease" (p.158, line 5947f), although this view is does not seem justified. We believe that substantially lowered concentrations of vaccine antibody concentrations and the associated increased incidence of infectious disease should be considered an adverse effect. The default procedures would require that EFSA applied an uncertainty factor when calculating tolerable intake levels. Because the study results reflect the average relationship between exposures and outcomes, the uncertainty factor would represent the differences in individual vulnerability. However, EFSA has chosen to leave out an uncertainty factor (p. 158, line 5945) and, in our view, does not provide proper justification. This choice is even more surprising, as EFSA relies on a NOAEL rather than a BMDL.	See the response to comment number 19.
	28	3.3.5.5 Impaired mammary gland development (MoA)	We also agree that the mammary gland appears to be highly susceptible, with human studies showing tendencies of shorter duration of breastfeeding among mothers with elevated PFAS exposures (36, 37), although this has not been replicated at lower exposures (38). As EFSA has not evaluated this outcome in any detail, and some important references were missed, we suggest that EFSA might apply a precautionary approach (3, 39) when considering such outcomes that likely represent true adverse effects. 36. Romano ME, Xu Y, Calafat AM, Yolton K, Chen A, Webster GM, et al. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. Environ Res. 2016;149:239-46. 37. Timmermann CAG, Budtz-Jorgensen E, Petersen MS, Weihe P, Steuerwald U, Nielsen F, et al. Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances. Reprod Toxicol. 2017;68:164-70.	Impaired mammary gland development and delay in involution of mammary tissue in the dams at weaning has only been studied for PFOA in experimental animals and has not been studied in humans. Studies on duration of breastfeeding in humans examined associations with maternal (adult) serum levels. Measured concentrations at adult age may differ substantially from past exposures so the mammary gland comparison made by the stakeholder has some important differences. In the Opinion on PFOS and PFOA (EFSA CONTAM Panel., 2018) it was concluded that the results reported in the human studies on breastfeeding were possibly influenced by reverse causation. This conclusion also holds for PFASs other than PFOS and PFOA. The opinion has now been updated to include studies on duration of breastfeeding (it was missing in the opinion sent for public consultation).

Stakeholder	Comment number	Chapter	Comment	EFSA response
			38. Rosen EM, Brantsaeter AL, Carroll R, Haug L, Singer AB, Zhao S, et al. Maternal Plasma Concentrations of Per- and polyfluoroalkyl Substances and Breastfeeding Duration in the Norwegian Mother and Child Cohort. Environ Epidemiol. 2018;2:e027. 39. Cordner A, De La Rosa VY, Schaider LA, Rudel RA, Richter L, Brown B. Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors. J Expo Anal Environ Epidemiol. 2019;29:157-71.	
Philippe Grandjean & Esben Budtz-Jørgensen	29	3.4. Critical Effects, dose-response assessment and derivation of a health-based guidance value	While we agree that immunotoxicity at the present time appears to be the critical effect, we note that other effects of major public health concerns have been covered rather superficially, thus giving the erroneous impression that the documentation is limited and that other adverse outcomes can be ignored. Our below comments on dose-response (DR) assessment and HBGV are focused on immunotoxicity.	Many outcomes have been studied in relation to PFAS exposure in human epidemiological studies. In the 2018 opinion on PFOS and PFOA, (EFSA CONTAM Panel, 2018), a detailed review of a large number of outcomes was performed. As mentioned in the introductory paragraphs of Section 3.3.4 the present opinion focussed on the four outcomes which were considered potentially critical in the 2018 opinion, and for these outcomes the literature review was also updated for PFOS and PFOA. Information on other outcomes in relation to other PFASs than PFOS and PFOA, was placed in Appendix J.
Philippe Grandjean & Esben Budtz-Jørgensen	30	3.4.3 Dose-response assessment	To compare with the EFSA findings, we calculated Benchmark Dose results for the sum of the four PFAS (PFOA, PFOS, PFHxS and PFNA) concentrations using first the complete Faroese data, then the decile version of the Faroese data that we had provided to EFSA, and finally using decile data from the study conducted by Dr. Abraham. For the individual level Faroese data, calculations were based on regression models with antibody concentrations as dependent continuous variables while serum-PFAS concentrations were included as independent variables along with potential confounders, as previously reported (10, 40). To achieve normally distributed residuals, antibody concentrations were log-transformed. We modelled the PFAS effect using a linear DR function [f(d) = βd], and a more flexible K-power model [f(d) = βd^K], with K≥1. As the DR relationship at low doses may differ from the one at higher doses, we also used a piecewise linear model, which allowed for a difference in slopes below and above the median exposure level (40). For the decile	Thank you, these results are noted. Regarding the use of BMD modelling, please see the response to your comment number 13.

<table>
<tr><th>Stakeholder</th><th>Comment number</th><th>Chapter</th><th>Comment</th><th>EFSA response</th></tr>
<tr><td></td><td></td><td></td><td>data (Dr. Abraham's and the Faroese), we used the same approach except for the confounder adjustment. For the Faroese data, this choice was appropriate, as the decile data had already been adjusted for covariates. The fit of the models was based on minus two times the log-maximum likelihood function [−2log(L)], where a smaller value indicates a better fit, as previously discussed (40). As a sensitivity analysis in the complete Faroese data set, we developed a low-dose threshold version of each of the DR models used. These conservative models were identical to the original DR model within the observed dose range, but with a flat (horizontal) slope below the lowest dose observed, again as previously described (10).

40. Budtz-Jorgensen E, Grandjean P. Application of benchmark analysis for mixed contaminant exposures: Mutual adjustment of perfluoroalkylate substances associated with immunotoxicity. PLoS One. 2018;13:e0205388.</td><td></td></tr>
<tr><td>Philippe Grandjean &

Esben Budtz-Jørgensen</td><td>31</td><td>3.4.3.1 Effects on the immune system</td><td>Faroes study
We saw a fairly close agreement between BMD results calculated based for the two versions of the Faroese data (individual data and decile data). The complete data from the Faroese study (2, 39) showed the following results for the age-5 serum total-PFAS concentrations (ng/mL) in regard to tetanus and diphtheria antibody concentrations at age 7 years, at two levels of Benchmark Response (BMR):
<table><tr><th></th><th></th><th colspan="2">BMR=5%</th><th colspan="2">BMR=10%</th></tr><tr><th>Antibody</th><th>DR-model</th><th>BMD</th><th>BMDL</th><th>BMD</th><th>BMDL</th></tr><tr><td>tetanus</td><td>linear</td><td>2.577</td><td>1.434</td><td>5.294</td><td>2.946</td></tr><tr><td>tetanus</td><td>K-power</td><td>2.577</td><td>1.434</td><td>5.294</td><td>2.946</td></tr><tr><td>tetanus</td><td>piecewise</td><td>1.516</td><td>0.673</td><td>3.113</td><td>1.383</td></tr><tr><td>diphtheria</td><td>linear</td><td>2.672</td><td>1.513</td><td>5.488</td><td>3.108</td></tr><tr><td>diphtheria</td><td>K-power</td><td>2.672</td><td>1.513</td><td>5.488</td><td>3.108</td></tr><tr><td>diphtheria</td><td>piecewise</td><td>1.222</td><td>0.632</td><td>2.511</td><td>1.298</td></tr></table>In these data, K was estimated to 1, and the K-power model therefore becomes identical to the linear model. In addition, we used a piece-wise linear curve that allowed different slopes below and above the median exposure. For these</td><td>These results are noted. Regarding the use of BMD modelling, please see our response to your comment number 13 above.

The opinion has been revised based on comments from you and several stakeholders and the BMD approach is now used to derive the reference point and related health-based guidance value for the sum of 4 PFAS.

Regarding comparison of the results shown here and the results derived by EFSA, the existing guidance on BMD modelling using PROAST was applied. The BMDL is based on the lowest obtained BMDL for 4 individual models and for the Abraham study resulted in a $BMDL_{10}$ that is close to somewhat in between the values generated by you using the K-power model. For your study, we did not obtain enough information to review the performed analyses.</td></tr>
</table>

Stakeholder	Comment number	Chapter	Comment	EFSA response

data, the slope at low doses was steeper and this model thus yielded somewhat lower BMD results. The curve fit calculated as -2log(L) showed that the different curves fit the data equally well.

Using the Faroese decile data, the BMD results were computed only for the linear and piecewise models. In these data, the difference between the BMD and its lower confidence limit (BMDL) was slightly greater. However, this was to be expected, as power is lost when using decile data. Still, the results did not change substantially, possibly because of the good precision of the decile data. The below table shows the results for the age-5 serum sum of PFAS concentrations (ng/mL) in regard to tetanus and diphtheria antibody concentrations at age 7 years:

		BMR=5%		BMR=10%	
Antibody	DR-model	BMD	BMDL	BMD	BMDL
tetanus	linear	2.110	1.290	4.334	2.649
tetanus	piecewise	1.965	0.683	4.037	1.404
diphtheria	linear	2.073	1.203	4.259	2.471
diphtheria	piecewise	1.243	0.531	2.553	1.091

In the complete and decile data, the linear and the more flexible piecewise linear model yielded results that were quite close, with BMDLs slightly above or below 1 ng/mL serum for a BMR of 5%. The results of piecewise linear models were generally lower than for the linear model, and the piecewise linear model also fitted data slightly better. Conservative models (with a flat DR curve below the lowest observed serum concentration) yielded BMDLs of about 10 ng/mL for a BMR of 5% (results not shown). These findings are in accordance with our previous publications (10,40), though now expressed in terms of the sum of the main four PFASs.

Abraham et al. study

From the decile results reported in the Abraham et al. study, as copied in the EFSA draft, we similarly calculated BMD results for sum of PFAS concentrations (ng/mL) in regard to HiB, tetanus and diphtheria antibody concentrations:

	BMR=5%	BMR=10%

Stakeholder	Comment number	Chapter	Comment	EFSA response
			Antibody DR-model BMD BMDL BMD BMDL HiB linear 2.107 1.148 4.327 2.357 HiB K-power 2.107 1.148 4.327 2.357 tetanus linear 4.213 2.326 8.654 4.778 tetanus K-power 31.10 13.51 36.16 19.82 diphtheria linear 3.575 1.864 7.343 3.829 diphtheria K-power 31.29 18.64 35.78 24.22 For tetanus and diphtheria in the Abraham data set, the K-power model fitted clearly better than the linear model. The fit shows a flat curve at low doses and a steep curve at elevated exposures. This finding agrees with the segmented regression analysis of the Abraham data in the EFSA draft. For HiB this tendency was not found, as K was estimated at 1, so that the K-power model and the linear model provided identical fits. For tetanus and diphtheria, the linear model showed BMDLs of approximately 2 ng/mL serum, while the K-power model showed higher levels. The HiB results were fairly close to the results obtained from the Faroese data, with a BMDL of 1.1 ng/mL at a BMR of 5%. For the Abraham data, the results of the piecewise model were difficult to interpret as the slope was positive at low doses. We therefore disregarded the model here. Thus, the German data had a narrower range of exposures and a smaller number of observations that yielded somewhat higher BMD results. Still, the BMDL data for the linear model were not materially different from the results obtained for the Faroese data. The Benchmark Dose approach therefore proved feasible and generated plausible results.	
Philippe Grandjean & Esben Budtz-Jørgensen	32	3.4.4. Derivation of a Health Based Guidance Value	We support EFSA's use of a transgenerational toxicokinetic model for derivation of a tolerable weekly intake limit and recommend comparison with the Minnesota model (4).	The CONTAM Panel examined the Minnesota model which applies only to PFOA. The outcome seems comparable with those from the modified Loccisano model and provides further support that the applied PBPK model is fit for purpose.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	33	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	We have already covered important weaknesses and uncertainties when using the NOAEL approach that EFSA has chosen. We strongly recommend using the BMD approach that is generally recommended by EFSA and national regulatory agencies. In the HBGV derivation, we wish to emphasize that increased transplacental transfer occurs in diabetic women and that the four PFASs may differ in their transfer into milk (5). These uncertainties have not been considered by EFSA.	The CONTAM Panel is aware of the weaknesses of the NOAEL approach and prefers to use BMD modelling. A particular issue is the availability of the individual data that allows the CONTAM Panel to perform this analysis. However, BMD modelling of the individual data from the study by Abraham et al. (2020) was performed by the authors, since these data could not be provided to EFSA. The CONTAM Panel was able to reproduce the results using data extracted from the graphs provided by the authors. BMDLs were obtained that could be used for the derivation of the TWI. Additional calculations were made to study the sensitivity of the PBPK model to variations in the model parameters. The PBPK modelling shows that the impact of the lactation on serum levels is much larger than the exposure *in utero*. Ideally the modelling is congener-specific but at this stage the preferred model only covers PFOS and PFOA, and assumptions had to be made about PFHxS and PFNA. However, also for PFOS and PFOA there is variation in the various parameters in the model which adds to the uncertainty of the approach.
Philippe Grandjean & Esben Budtz-Jørgensen	34	3.6.5 Summary of Uncertainties	We recommend that our above comments be taken into regard.	Noted.
Philippe Grandjean & Esben Budtz-Jørgensen	35	4. Conclusions	BMD results can be feasibly used for determining tolerable intake levels. The default linear DR curve is appropriate after log transformations. As the adverse effects are seen in children, the corresponding maternal intake must be assessed, as EFSA has done. We support EFSA's use of a transgenerational calculation that has similarities to the model developed by the state of Minnesota (4). This approach takes into consideration the very substantial developmental exposure that occurs through placental passage and also through excretion into human milk (5). However, we note that there is some uncertainty involved, as greater placenta passage occurs in diabetic mothers (6) and because some data on PFHxS suggests a lower transfer into human milk than for, e.g., PFOS (5).	See responses to previous comments, i.e. comment number 13 and 33 for BMD modelling and comment numbers 10 and 33 for PBPK modelling.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Philippe Grandjean & Esben Budtz-Jørgensen	36	5. Recommendations	The present comments raise a number of issues that need to be considered by EFSA in order to revise and strengthen the conclusions. In general, we find that most of the conclusions are reasonable, but we are missing more detailed justification that relies on the strength of published evidence and also a more complete coverage of the human studies. Thus, fewer than one-quarter of the publications cited in our comments were included in EFSA's list of references. Our main concern is that reverting to the NOAEL approach is a serious mistake that ignores EFSA's own recommendations and needs to be corrected. Our tables show the usefulness of Benchmark Dose calculations and the use of DR models. Although we have not entertained a complete and thorough review of the draft opinion, we hope that the above comments and suggestions may be of help to the Agency in developing a final opinion.	The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the risk assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the risk assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N). For BMD modelling, see reply to comment numbers 13 and 33.
Philippe Grandjean & Esben Budtz-Jørgensen	37	Appendix H-Immunotoxicity	Our main comments are properly covered above.	Noted
Philippe Grandjean & Esben Budtz-Jørgensen	38	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex A of this Technical report (Content as provided in comment number 31).*	-

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	39	Summary	BLV would like to compliment EFSA, the CONTAM Panel and its PFAS Working Group for the comprehensive work done and to thank for the opportunity to comment on the draft opinion. According to the terms of reference, the risk should have been assessed for PFASs related to their presence in food. 27 PFASs were considered in this opinion. Chronic exposure could be estimated for 17 PFASs. A TWI was derived for the group of "only" four PFASs (PFOA, PFNA, PFHxS, PFOS). It should be explained why no risk assessment was performed for the other 13 PFASs. Which were the reasons that no TWI values could be derived for these 13 PFASs? Does EFSA consider the toxicity database insufficient for a hazard assessment? Will these PFASs be evaluated in a separate EFSA opinion in the future?	All 27 PFASs were reviewed and it was concluded that immune effects and impaired mammary gland development were the most sensitive endpoints. Data on immune effects are only available for some PFASs and for the mammary gland only for PFOA. In principle, other effects could have been used to assess the risk of other PFASs but it was observed that for the 4 PFASs included in the TWI, other effects in animals occur at much higher exposure than effects on the immune system and mammary gland. Using other such endpoints could suggest that current exposure to these other compounds is not of concern. Based on structural similarities and shared effects on liver and thyroid hormone levels, other PFASs are suspected to cause similar effects as the 4 PFASs Therefore, it is important that more data become available for the critical endpoints and on relative potencies. Also the accumulation potential of the various PFASs should be taken into account since this is important at background exposure. At this stage, these other PFASs were not included or detected in the critical human studies. A statement that other PFASs may cause similar effects is now added in the opinion. In addition, it is important to obtain more data on the levels of other PFASs in food to decrease the uncertainties in the exposure assessment. LOAEC and NOAEC are now spelt out the first time they are used in the opinion.
			Line 195: "LOAEC" and "NOAEC" should be spelt out when first used and added to the list of abbreviations.	
Federal Food Safety and Veterinary Office	40	1.3.1. Chemistry and Synthesis	Table 1: n:2 fluorotelomer alcohols (n:2 FTOHs): Beside 8.2 FTOH also 6:2 FTOH is used as principal raw material to manufacture FTOH-based products. The question is why 6:2 FTOH has not been considered in this opinion.	The selection of PFASs in this opinion is based on the 27 PFASs that were the subject of the EFSA 2012 report: *EFSA (European Food Safety Authority), 2012. Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012;10(6):2743. 55 pp. https://doi.org/10.2903/j.efsa.2012.2743.*

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	41	1.3.2.2. Analytical methods for determination of PFASs in biological samples	Line 647-8: "Clean up is often done using dispersed graphitized carbon with glacial acetic acid or by filtration (Salihovic et al., 2013)." Clean up with dispersed graphitized carbon and glacial acetic is only one clean up procedure among many others, why is it mentioned in an extra sentence? This sentence may be skipped and if really necessary the procedure may be added in the previous phrase.	The CONTAM Panel agrees with the comment and the sentence has been deleted.
Federal Food Safety and Veterinary Office	42	1.3.5. Previous risk assessments	Line 1078-9: "In 2018 the EFSA CONTAM Panel established a tolerable weekly intake (TWI) of 13 ng/kg bw per week for PFOS and 6 ng/kg bw per week for PFOA." It should be mentioned here that these two TWIs should be regarded as provisional. The International Agency for Research on Cancer (IARC) classified PFOA as group 2B, a possible human carcinogen. IARC (2017). Some chemicals used as solvents and in polymer manufacture. IARC Monographs on the evaluation of carcinogenic risks to humans. Perfluorooctanoic acid. Volume 110:37-110	The CONTAM Panel agrees with the comment and a sentence has been added to Section 1.3.5, that refers to the explanatory note in the 2018 Opinion (EFSA CONTAM Panel, 2018). With respect to PFOS and PFOA in this Opinion, risk assessments are reviewed that have been published after the 2018 EFSA CONTAM Panel opinion. This is stated at the beginning of the respective chapter. The IARC assessment (available 2016 and published 2017) is summarised in the EFSA CONTAM Panel (2018) Opinion. In addition, we refer to the IARC assessment in this Opinion in Section 3.3.4.8. (Carcinogenicity outcomes).

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	43	3.1.4.1 Migration from food contact materials, including non-stick coatings used on cookware	This subchapter is quite short and should go into more detail. Additional information on content and migration from food contact materials are available in two recent reports by RIVM (2018) and TemaNord (2017). According to the RIVM report, considerable amounts of PFCAs and FTOHs can migrate from paper and board food contact materials into food. This was demonstrated in diverse migration tests. PFAS exposure from packaged food and fast-food wrapped in coated paper and board with grease-resistant properties should be more systematically assessed in this opinion. RIVM (2018). Per- and polyfluoroalkyl substances (PFASs) in food contact materials. RIVM Letter report 2018-0181. doi 10.21945/RIVM-2018-0181. TemaNord (2017). PFAS in paper and board for food contact. Options for risk management of poly- and perfluorinated substances. http://dx.doi.org/10.6027/TN2017-573.	Thank you, neither of these reports was identified in our literature search for this topic which focussed on articles published in peer reviewed journals between 01 2007 and 09 2018. The CONTAM Panel noted that these detailed assessments on migration did not contradict the conclusions drawn in the Opinion, and that the use of PFASs in food contact materials is likely to contribute to overall human exposure, but that the exposure from this route will be small compared with other sources. The reports also support the CONTAM Panel recommendation that: 'More studies on the effect of cooking and food processing, in particular in relation to transfer to food from food contact materials that contain PFASs, are needed.'
Federal Food Safety and Veterinary Office	44	3.2.1. Current exposure assessment	Line 1736: In Table 6, a very high mean dietary exposure for infants with PFDoDA (and to a lower extent with PFNA) is given. It is probable that the exposure calculated for PFDoDA is the same type of "artefact" caused by a combination of a low number of positive samples in combination with an infant diet consisting of a very limited number of products as already commented for PFNA exposure (see lines 1784 ff). Therefore PFDoDA should be included into this comment.	The CONTAM Panel agrees that PFDoDA should be included in the comment, and a sentence has been added in Section 3.2.1.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	45	3.3.2.1. Selection of biomarker and appropriate matrix for assessing internal dose	Line 2446-8: "In general, the detection frequency in urine was very low except for PFPeA which was found above the LOQ in 70% of the samples with a mean concentration of 2.34 ng/mL …" It should be indicated that the high results reported for PFPeA and PFHpA may be due to the elevated exposure to perfluorinated compounds in this South Korean city. Furthermore, it is remarkable that the concentrations for all three detected PFCAs were higher in urine than in serum, which is in contrast to the other studies given. We suggest to also mention a study published by Kato et al in 2018 (Chemosphere 209 (2018), 338-345) here. In this study paired human serum and urine (n=50) were investigated: in urine, only PFBA was detected while in serum an elevated number of PFCAs and PFSAs were found.	The CONTAM Panel agrees to include the study by Kato et al. (2018), and this has now been done.
Federal Food Safety and Veterinary Office	46	3.3.2.5. Levels in blood from populations with elevated drinking water exposure	In this subchapter, a recent publication about PFAS serum levels in the Veneto Region, Italy has not been cited: Ingelido et al. (2018). Biomonitoring of perfluorinated compounds in adults exposed to contaminated drinking water in the Veneto Region, Italy. Environment International, 110, 149-159. There is a more recent publication covering the Veneto Region by Pitter et al. (2020). From 18'345 subjects 14-39 years of age, the median PFOA serum level was 44.4. ng/mL, and the 95th percentile 189.7 ng/mL and maximum 1'400 ng/mL. Pitter et al. (2020). Serum levels of perfluoroalkyl substances (PFAS) in adolescents and young adults exposed to contaminated drinking water in the Veneto region, Italy: A cross-sectional study based on a health surveillance program. EHP 128.	The study by Ingelido et al. (2018) was already included in Appendix B, but the CONTAM Panel agrees that this study should also have been mentioned in Section 3.3.2.5. The study has now been added. The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N).

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	47	3.3.2.6 Factors that may have an impact on the internal dose of PFASs	Lines 2592-6: There are two new studies on PFAS trans-placental transfer in humans available that could be mentioned here: Gao et al. (2019). Prenatal exposure to per- and polyfluoroalkyl substances (PFASs)and association between the placental transfer efficiencies and dissociation constant of serum proteins-PFAS complexes. Environ. Sci. Technol. 53:6529–6538. Li et al. (2020). Transplacental transfer of per- and polyfluoroalkyl substances identified in paired maternal and cord sera using suspect and nontarget screening. Environ. Sci. Technol. https://pubs.acs.org/doi/abs/10.1021/acs.est.9b06505	Please see response to comment number 46.
Federal Food Safety and Veterinary Office	48	3.3.3.7 Long-term toxicity and carcinogenicity	Line 3982-393: "The CONTAM Panel has not identified relevant new carcinogenicity studies for PFOS and PFOA in experimental animals". In the meantime, the new NTP 2-year carcinogenicity study on PFOA in rats was published (Draft NTP Technical Report 598, 2019). It came to the conclusion that "there was clear evidence of carcinogenic activity in male SD rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas. … There was some evidence of carcinogenic activity of PFOA in female SD rats based on the increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure." Massive increase of acinar cell adenoma or adenocarcinoma (combined) was observed in male rats already at the lowest concentration tested (20 ppm) and at higher concentrations.	Tumour formation in liver and pancreas in PFOA-treated animals was documented and discussed in detail in the Section on the mode of action (3.3.5.5.) in the EFSA CONTAM Panel Opinion (2018). The NTP Technical Report 598, 2019 was published after the literature deadline of the current opinion and has therefore not been included. Furthermore, as it is still draft, it could not have been used in the assessment as NTP state on the report that it is distributed solely for the purpose of predissemination and it is not for attribution.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	49	3.3.5. Mode of action	The carcinogenic effects in liver, pancreas and uterus from the NTP carcinogenicity study on PFOA in rats (NTP 2019) need further MOA elucidation and interpretation regarding their human relevance in this chapter. See our comment in 3.3.3.7.	The MoA underlying the tumor formation in liver and pancreas in PFOA-treated animals and the human relevance has been discussed in detail in Section 3.3.5.5 in the 2018 Opinion (EFSA CONTAM Panel, 2018). See also reply to comment 48.
Federal Food Safety and Veterinary Office	50	3.4.1 Critical effects	Lines 5668ff: "Although the association with increased cholesterol was observed in a large number of studies, the CONTAM Panel now considers the uncertainty regarding causality larger. This is primarily due to a postulated biological process around the enterohepatic cycling of both PFASs and bile acids, the latter affecting serum cholesterol levels." Based on the text it is difficult to follow the argumentation why the critical endpoint from the EFSA opinion 2018 on PFOS and PFOA (increase of serum cholesterol) is no longer considered for the TWI derivation. It is recommended to give some more background information and references on the "postulated biological process around the enterohepatic cycling" in the opinion.	The CONTAM Panel has expanded the considerations of the uncertainty regarding causality in the critical effect Section (3.4.1) of the Opinion.
Federal Food Safety and Veterinary Office	51	3.4.2 Mixture Approach	Line 5728-9: «In the German study, at the LOAEC the antibody titres for Hib had already decreased by more than 50%.» According to Table K.1. in Appendix K, in the fourth quantile Q4 (= LOAEC), the mean of antibodies against Hib is 1.38. Compared to the mean of 1.92 in the first quantile Q1 (= control group), this is a decrease by 28.1% and not by more than 50%. Also the antibody titres for HiB in the fifth quintile Q5 are decreased by only 22.4% that means never exceeds the 50% level. Is it possible that of the mean and SD are on a logarithmic scale in Table K.1.? Then this information is missing in the table caption. In addition, it is recommended that the % decrease of antibody titres compared to the first quantile Q1 is given in Table K.1.	The mean and SD are indeed on a Log scale. This has been clarified. The decrease compared to Q1 is now reported as suggested. In the Grandjean et al. (2012) paper, the protective level was defined as values below 0.1 IU/mL for tetanus and diphtheria antibodies.

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			Line 5730-2: "In that study, the proportion of children having vaccination titres below the protective limit after vaccination was also increased at higher PFAS levels." Which limits of antibody titers after vaccination can be considered as protective in the studies by Greandjean et al. (2012) and Abraham et al. (2020)?	Abraham et al. (2020) discuss protective levels for tetanus only: 'according to the World Health Organization (WHO), "*the minimum amount of circulating antitoxin that in most cases ensures immunity to tetanus is assay-specific. Within in vivo neutralization tests, modified ELISAs or bead-based immunofluorescence assays, concentrations at or exceeding 0.01 IU/mL are usually considered protective against disease, whereas antitoxin concentrations of at least 0.1–0.2 IU/ mL are defined as positive when ELISA techniques are used for the assessment" (WHO, 2018).*'
Federal Food Safety and Veterinary Office	52	3.4.3 Dose-response assessment	Line 5856: "… but also milk levels decrease over the lactation period" and together with line 331-4: "This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant associated with a decrease in vaccination response." It would be useful to graphically show the curve of the levels of PFOA (0.33 ng/kg bw per day intake) and PFOS (0.83 ng/kg bw per day intake), respectively, in milk during the one year lactation period calculated in the PBPK model.	This information showing the exposure of the infant per kg bw, was shown at the Stakeholder meeting and is now added to the Appendix on PBPK modelling.
Federal Food Safety and Veterinary Office	53	3.6.1 Uncertainty in exposure estimates	Line 6021-4: "Whilst PFASs released from materials in contact with food may not be a significant contributor to exposure on a population basis, this may have a disproportionately large impact on some individuals. It is not known to what extent such release is covered by the occurrence database. This might lead to an underestimation of the exposure." This refers back to subchapter 3.1.4.1 (see our comment there). It remains unclear how the exposure via food packaging was quantitatively assessed and how the uncertainty was analysed in this opinion. In the focus is fast-food wrapped in coated paper and board with grease-resistant properties. According to BBC news (2016), one in six young people eat fast food 'twice a day'. This exposure pathway may therefore have an impact on a considerable percentage of the population.	The uncertainties highlighted in the Opinion as described here, are the basis (in part) for the recommendation that '*More studies on the effect of cooking and food processing, in particular in relation to transfer to food from food contact materials that contain PFASs, are needed.'*

Stakeholder	Comment number	Chapter	Comment	EFSA response
Federal Food Safety and Veterinary Office	54	4.2 Hazard Identification and characterisation (Conclusions)	Line 6345-6: It should be mentioned here that PFOA causes tumours in liver, pancreas and uterus in rats (see NTP study, 2019). Line 6398-9: "Epidemiological studies show no evidence for carcinogenicity of PFOS and PFOA in humans, as concluded in the 2018 opinion (EFSA CONTAM Panel, 2018)." IARC (2017) assigned PFOA to group 2B as being possibly carcinogenic to humans. It identified a positive association of PFOA exposure and cancers of the testis and kidney in humans. In the highest tertile of PFOA exposure relative to non-chemical factory workers, liver cancer mortality was almost seven-fold increased and lymphatic and hematopoietic cancer mortality five-fold increased in an occupational cohort study in Italy (Girardi et al. 2019). Elevated PFOA levels in the body were associated with greater risk of non-Hodgkin lymphoma and kidney, testicular, prostate, and ovarian cancers in the C8 Health Project (Barry et al. 2013). Girardi P. and Merler E. (2019). A mortality study on male subjects exposed to polyfluoroalkyl acids with high internal dose of perfluorooctanoic acid. Environ. Res 179, 108743.	Please see response to comment number 48. As the comment notes, the IARC monograph categorised PFOA in group 2B (possibly carcinogenic to humans). The data from human studies provided 'limited evidence' for carcinogenicity in humans. This was based on about a dozen epidemiological studies, and there was some evidence for increased risk of cancer in the kidney and testis after exposure to PFOA. None of the studies reviewed by IARC showed, however, increased risk of liver cancer, lymphoma or leukemia. The study by Girardi and Merler (2019), (study published after the literature search deadline for this effect) examining workers with high exposure to PFOA, is interesting, but this single study is not sufficient to change the overall conclusion on carcinogenicity in humans.
Federal Food Safety and Veterinary Office	55	Appendix K – Additional information on the study from Abraham et al. (2020)	Is it possible that of the mean and SD are on a logarithmic scale in Table K.1.? Then this information is missing in the table caption. It is recommended that the decrease of antibody titres are given in percentage compared to the first quantile. See also our comments in 3.4.2.	The titres are based on a $\log_{10}$ scale and this information is now provided in the table legends. In addition, the % decrease in the absolute titres is provided.
Center for Research on Ingredient Safety Michigan State University (1)	56	3.3.1.3. PBPK modelling	2399 – 2464 Part 1, Section 3.3.1.3, is poorly written and as currently presented it is not possible to follow the discussion. Since suppression of antibody titers to vaccinations, specifically those <1 year of age, was identified as one of the most sensitive sequela of PFAS exposure, there was a need for a	Most of the publications dealing with PFAS PBPK modeling are based on PFOS and PFOA and have been described in the previous 2018 EFSA CONTAM Panel Opinion. As stated in the first sentence of Section 3.3.1.3, the objective here was not to discuss in detail the literature published on PFAS PBPK models, but to

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			human PBPK model that could described transfer of PFOA/PFOS compounds from breast milk to neonates, as the neonatal level of the compounds of interest was critical to the assessment. Although a number of studies are discussed in this section, Chou and Lin (2019), Kim et al. (2018), Kim et al. (2019) – does not appear in reference list, and Loccisano et al., 2011, it seems that none of the published models described human breast milk transfer of the PFOA/PFOS compounds. It appears that the Loccisano model was used as a starting point and modified to provide the needed predictive capability. The Berkeley Madonna code for this unpublished model is provided in Appendix M. Importantly and as presented, it is not clear exactly how these models that are referenced were actually used, or even if they were used, in developing the final human breast milk model. Again, since human breast milk transfer of the PFOA/PFOS compounds is critical to this assessment, there is a need for a very clear and precise explanation (i.e., clear roadmap) on exactly how these models that are referenced were used and likewise how the final PBPK modeling was derived. In addition to the deficiency described above, the discussion of PBPK modeling in Section 3.3.1.3. does not inspire confidence in the contribution of PBPK modeling to the development of this EFSA document. For example, starting on line 2349, page 73, it is stated: The developed PBPK model was extrapolated to humans, considering the interspecies differences of physiological and chemical-specific parameters using the following allometric equation: k human = k rat (BWhuman /BWrat) −0.25 This equation describes standard allometric scaling of the first order rate constant k (units of time-1) from rats (krat) to human (khuman). However, though saturable transporters are important determinants of PFOA/PFOS	briefly update the description provided in the previous EFSA CONTAM Panel Opinion, with a focus on PFASs other than PFOS and PFOA. Studies published by Chou and Lin (2019), Kim et al. (2018), Kim et al. (2019) are included in Section 3.3.1.3, but the latter was missing in the reference list provided at the end of the opinion. This has now been added. The contribution of PBPK modeling to the opinion, taking into account the maternal-infant transfer is described in Appendix M and not in Section 3.3.1.3. As described in Section 3.3.1.2 and in Appendix C, the CONTAM Panel agrees that interactions with transporters are important determinants of PFOA/PFOS toxicokinetics. The gastrointestinal reabsorption process as well as the renal tubular reabsorption are included in the model published by Kim et al. (2018) regarding PFHxS and explicitly mentioned in the paragraph describing this study (L. 2326-2337)

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			kinetics, scaling of saturable processes have a different mathematical form that is not described. Likewise neither is scaling of compartmental (tissue) volumes described. The text gives the impression that the equation for scaling of first-order kinetics that is presented is sufficient to describe allometric scale-up of a PBPK model from rats to humans. It is not. One is left wondering how familiar the author(s) of this section is with technical aspects of PBPK modeling. 5870 - 5907 Figures 12 through 15 (pp. 155 – 157) show simulations obtained with the PBPK model. The legend for Figure 12 states: Figure 12: Serum levels in a woman exposed in utero, via breastfeeding for 12 months and subsequently via food intake for 49 years to 0.33 ng/kg bw per day of PFOA. The level peaks at 9 months of breastfeeding around 16 ng/mL, decreases and then increases to a steady state level around 3.7 ng/mL. No indication is provided that this is a prediction of the PBPK model! A reader unfamiliar with PBPK modeling might easily assume that actual data were being presented. This deficiency applies to all these figure legends (Fig. 12 – 15)	The figure legend has been updated to include this information.
Center for Research on Ingredient Safety Michigan State University (1)	57	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	6127 – 6206 In Section 3.6.4.3 on PBPK Modeling, uncertainties in dose-response assessment and HBGV derivation are discussed (page 162). While this is an important section, it does not consider basic quality control information for PBPK models. When these kinds of models are used for derivation of an exposure standard, one would expect assurance about the integrity of the model code. This could be provided by statements about units checking and mass balance checking. This important information should be provided.	Basic quality control (mass balance, uncertainty, sensitivity) has now been added to Appendix M. Mass balance was not provided in the original model by Loccisano et al. (2011).

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Center for Research on Ingredient Safety Michigan State University (1)	58	Appendix M - PBPK Modelling	10787 - 10793 Appendix M – PBPK modeling (Part 2, page 444). Under Model Validation (line 10787), the following text: The model was applied in a case study of human individuals living in Little Hocking (Ohio, USA) and Arnsberg (Germany), and exposed to relatively high concentrations of PFOA through consumption of drinking water. For PFOS data from occupational exposure were used. The result was a PBPK model reasonably capable to estimate the concentration of PFOS and PFOA in the human body. Coding and simulations for both the PFOA and PFOS models was performed in the Berkeley Madonna program (Macey et al., 2000). One would expect to see a figure showing both the data and the simulation. A goodness-of-fit measurement would also be appropriate. None of these are provided. 10890 - 10939 Berkeley Madonna code for the PBPK model (Part 2, page 447). Commenting is inconsistent. All parameters and equations should have units specified.	Model prediction is already described in Loccisano et al. (2011). The authors achieved acceptable prediction (e.g. with the ratios of predicted to observed concentrations all within a factor of 2 according to WHO/IPCS, 2010 guidance) between the observed experimental data and the model predictions in humans. However, model evaluation (model prediction, uncertainty, sensitivity) has now been added to Appendix M according to the WHO/IPCS 2010 guidance. Data used by Loccisano et al. (2011) in evaluating the human model, consisted of serum measurements in residents from Little Hocking (Ohio, USA) who were exposed to contaminated drinking water. They also used data from Red Cross blood donors. Additional comparison has been added by the CONTAM Panel (model prediction in children, Fromme et al. (2010)). In conclusion, the models are able to reproduce the experimental data reported in adult and in children. The prediction of the model (with additional input, e.g. BW curve) is acceptable, with the ratios of predicted to observed concentrations within a factor of 2.
Center for Research on Ingredient Safety Michigan State University (2)	59	Summary	306-316 It is worrisome that the COMTAM panel has selected an unpublished investigation to serve as one of the most important studies on which the EFSA PFSA scientific opinion is based. Specifically, this is the Abraham et al. 2020 study of infants from Germany showing an inverse association between serum levels of PFAS and antibody titres to haemophilus influenzae type b, diphtheria and tetanus. Although some of the results from the paper are provided in Appendix K, this information is not sufficient to evaluate the quality of the study and the conclusions that are drawn. Because the Abraham et al. 2020 study is central to the PFAS risk assessment, it is difficult to evaluate the overall conclusions in this document.	See response to comment 15 regarding the inclusion of data from Abraham et al. (2020) and their inclusion in the draft Opinion. Furthermore, the paper was published during the public consultation *(Archives of Toxicology, 94:2131-2147*).

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Center for Research on Ingredient Safety Michigan State University (2)	60	3.3.4.4.2 Vaccination response	It is worrisome that the COMTAM panel has selected an unpublished investigation to serve as one of the most important studies on which the EFSA PFSA scientific opinion is based. Specifically, this is the Abraham et al. 2020 study of infants from Germany showing an inverse association between serum levels of PFAS and antibody titres to haeomophilus influenzae type b, diphtheria and tetanus. Although some of the results from the paper are provided in Appendix K, this information is not sufficient to evaluate the quality of the study and the conclusions that are drawn. Because the Abraham et al. 2020 study is central to the PFAS risk assessment, it is difficult to evaluate the overall conclusions in this document. In addition to the unpublished German infants study (Abraham et al. 2020), a second crucial series of studies that are cited and also are critical to this assessment as supporting a linkage between PFAS exposure and a decrease in immune competence are measurements of antibody titres to vaccinations in Faroe Island children by the Grandjean investigative team. It is noteworthy that the Grandjean team's conclusions with PFAS exposure are remarkably similar to a prior series of high impact publications describing decreased antibody titres to diphtheria and tetanus toxoids in Faroe Islands children due to prenatal and postnatal PCB exposures (Heilmann et al. 2006, Heilmann et al. 2010). With the exception of two sentences provided in this report (please see below) the influence of PCB as a confounder on immuncompetence in this population is not addressed. Specifically, on line 4353 p120, it states: "Concerning possible confounding by other co-exposures, PCBs in maternal samples and offspring samples at age 5.0 years showed a weak correlation with individual PFASs. Adjustments for these co-exposures had no impact on the effect estimates"	See response to comment 59. The method used for adjusting for PCBs as potential confounder in the Grandjean et al. (2012) study is well described. The use of multivariate regression for confounder control is a well-accepted and scientifically sound method. It is beyond the scope of the Opinion to provide detailed explanation on the technical aspects of multivariate regression and confounder control. Concerning confounding by PCBs, for PCBs to be a confounder it has to be a strong predictor of both PFAS concentration and antibody concentrations. It is clear for previous studies from the Faroes Islands that PCBs are associated with antibody response. That on its own is not enough because if PCBs and PFAS are uncorrelated or weakly correlated no or limited confounding can occur. As shown in the JAMA (2012) publication (in the supplementary material) PCBs and PFAS were only weakly correlated and adjustment had no impact on the effect estimates meaning that PCBs cannot be a strong confounder. This is further supported by the fact that PFAS concentrations in the Faroe Islands around the time the study was conducted, were similar compared to levels reported for other European countries, while exposure to PCBs was much higher (largely driven by consumption of marine mammals).

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			Based on the significance of the prior Grandjean PCB publications showing decreased antibody titres to vaccinations in Faroe Islands children, it is crucial the authors of this section provide a thorough and critical discussion in this section explaining the specific methods and adjustment the Grandjean team used to rule out confounding due to PCB exposure in the more recent PFAS studies, and why these methods and adjustments are scientifically appropriate.	If PCBs would have been a confounder then the effect estimate for PFASs would have been reduced substantially when accounted for in statistical models. As can be seen in supplemental material in the Grandjean et al. (2012) paper, this was not the case. In the Abraham et al. (2020) study multiple co-exposures were also accounted for. Here again PCBs were associated with antibody response, but this exposure was unrelated to PFASs and adjustment had again no influence. Overall, similar associations between PFASs and antibody response have been reported from several countries including the Faroe Islands, Denmark, Norway and Germany, where fish intake and exposure to PCBs are different. This is again inconsistent with confounding from PCBs.
German Environment Agency	61	Summary	L 332-334, P 8 It should be specified for which part of the population the TWI should be applied. The wording "As a result, the higher exposure of breastfed infants is taken into account in the derivation of the TWI, and this TWI is not applicable for infants" is not clear.	In principle HBGVs are derived for the most sensitive part of the population and as such protect also the rest of the population. It should be noted that there are insufficient data on the sensitivity of other age groups considering effects of PFASs on the immune system. Furthermore, these contaminants are associated with several other potential effects at similar serum levels. Breastfeeding is important for infant health and should not be affected by the presence of contaminants. Therefore, the TWI was derived in such a way that exposure of infants via the mother does not result in increased risk. Since the higher exposure is already taken into account, the exposure of infants via breastfeeding or other food should not be compared with the TWI.
German Environment Agency	62	1.3.5. Previous risk assessments	L 1108, P 29 The citation is wrong, it should be: In 2017, after consulting the German Drinking Water Commission, the German Environment Agency recommended the following drinking water guide values….. (Bundesgesundheitsblatt 2017, 60:350-352).	The CONTAM Panel agrees to change as suggested.
			Please add: The German Human Biomonitoring (HBM) Commission at the German Environment Agency has derived	The CONTAM Panel agrees and added the provided further information.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			HBM-I and -II values for the health related assessment of human biomonitoring data for PFOA and PFOS. The HBM-I value corresponds to the concentration of a substance in human biological material below which, according to the current status of assessment, no adverse health effects are to be expected, it is set to 2 ng PFOA/mL and 5 ng PFOS/mL in blood serum or plasma, respectively (Bundesgesundheitsblatt 2018, 61(4):474–487). In contrast to the HBM-I value, the HBM-II value corresponds to the concentration of a substance in human biological material which, when exceeded, may lead to health impairment which is considered as relevant to affected individuals. The HBM Commission established HBM-II values for women at child-bearing age of 5 ng PFOA/mL blood plasma and 10 ng PFOS/mL blood plasma such as for all other population groups of 10 ng PFOA/mL blood plasma and 20 ng PFOS/mL blood plasma. Both, the HBM-I and the HBM-II values for PFOA and PFOS are based on the assessment of the population-related risk of changes in selected effect indicators. (Bundesgesundheitsbl 2020, 63:356–360) https:// doi.org/ 10.1007/ s00103- 020- 03101-2 Translation of the German version in Bundesgesundheitsbl 2020, 63:356–360 available under https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission/opinion-of-the-human-biomonitoring-commission-hbm	
German Environment Agency	63	3.3.2.2 Time trends	2486, P 76 Please add: A new study, analyzing more current samples of the German Environmental Specimen Bank, showed slightly lower mean concentrations for PFHxS for the years 2009 to 2019 (2019: AM = 0.63 ng/mL, median 0.53 ng/mL) (Goeckener et al. 2020, in preparation). L 2493, P 76	The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N).

Stakeholder	Comment number	Chapter	Comment	EFSA response
			Goeckener et al. (2020) show a decreasing trend for PFNA starting in 2006 with mean and median concentrations in 2019 being 0.38 ng/mL.	
German Environment Agency	64	Appendix B – Biomonitoring	see uploaded file *The attachment submitted by this commenter during the public consultation is listed and available in Annex B of this Technical report.*	The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N).
Carlotta Bargossi	65	*(not specified)*	The draft is a long summary of important results that help us understanding something about PFAs, some outcomes on dietary exposure, some hipothesys on methabolic pathways, also evidences on their interaction with biologic processes. These compounds behave, like other POPs, as endocrine disruptors as they can module immunosystem response and interfere with lipids methabolism (as well as steroid hormones). They are a risk for our health and fixing a No Adverse Effect Level is a primary goal. However it's not clear how data presented in Annex A, B, C, and all through the draft (transfer from feed to food, biomonitoring, epidemiological studies, etc..) have contributed to the implementation of PBPK model, and eventually to the proposal of the TWI. Starting from some conclusions: line 6471 This TWI should prevent that mother reach a body burden that results in levels….in milk that can be dangerous for breastfed infants. Levels come from two studies, the German one and the Faroe Islands one, which correlate NOAEC serum levels at	Thank you for your comments. A number of uncertainties were identified and recommendations have been provided in order to improve the assessment. The derivation of a TWI will be maintained.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			the age of 1 year and 5 years to inverse association with antibody titres for some diseases. The TWI, in nutshell, is the result of these experiences and the PBPK model. This model is made up of big data set, whose confidence is not discussed. The data input for the model are supposed to be "the best fitting" ones to represent the complexity of several biological processes like transfer of Pfas from food to plasma/serum and to milk. Surely they are, but Appendix M is quite hazy; I think that PFAs question is quite recent and data in our hands are often used, let's say forced, behind their value. My contribution comes from analitical experience in lab activities. As a lab technician, I suggest all the difficulties of dealing with rather new contaminants, whose knowledge is poor, lacking of technologies up to the aim. ARPA labs have different equipments and ISPRA report 305/2019 shows a certain disomogeneity in analytcal performances. In surface waters, the easiest samples if compaired to plasma or other biological matrix, few steps are needed before instrumental analysis; preconcentration (250 times and more) is the most important one, because of very low detection limits imposed by law; SPE (solid phase extraction) needs very well trained operators. If samples are clean and you can skip some steps, you may have filtration problem, because perfluorocompounds are wide spread in lab materials, and blanks are challenging; in any case high resolution detectors are necessary and long experience with trace analysis. In this regard, labs complain lack of Proficiency Test at very low concentration to prove and check their technical skill. I think that growing interest in this matter led to an increase in PFAs analysis and a wide spread provision of data, some of which totally unreliable.	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			Results of monitoring plans don't come from statistic analysis of large amount of data, but are driven by limits of the law; PFOS is present in 50% and more of water samples under analysis, because Law limits are 10 times lower than for other compounds. Most of the difference is due to false positives. Statistic approach is crucial to understand the extent of PFAS question, in order not to over or underestimate it; since the results are often < LOQ, the choice of LB or UB criterion makes the difference. In surface water, PFAS are seldom or weakly bound, but in serum and plasma PFAs may be associated with albumin and other protein; I think various analitical problems can affect the result. Also milk analysis is a quite long procedure, starting with precipitation of proteins and proceeding with similar steps. In 2013 a group of researchers (Barbarossa et al.) has investigated Pfas level in human milk provided by primiparous mothers (PFOS ranges from 100 to 300 ng/l, higher concentration in primiparous mothers.. Correlation between blood Pfas and milk concentration have also been found...serum or plasma ?… I think this line of research can be very promising. Again, 27 ng/ml in 1 year child serum is consistent with data in milk? For sure, informations on food consumption must be improved and FoodEx system will help to homogenize data. Data in Annex A, like INRAN SCAL (2005-2006) are quite old and have beenconceived for other aim.	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			The representativity of italian dietary is poor, both for number of families (1329) taken into account, and for composition of the sample. Children under 3 years are quite ignored; breast feeding mothers are only 10 (but these categories are those for whom the WTI has been thought). Emilia Romagna is represented with less than 100 families, living in the western region (RE), while people living in the coastal region where fish consumption is greater, have not been considered. (Further investigation in last years proved that farmed fish is healthier than caught fish, and in general wild animals are more contaminated than breed ones, owing to environmental pollution.) We know very little about what people do eat. So, Goethe paradox says that knowledge is like a sphere, the more the kwnoledge, the more the surface touching the unknown. In conclusion, I think that the proposal of a TWI is somehow ambitious, and pretty useless, at this early stage of investigation.	
Carlotta Bargossi	66	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex C of this Technical report (Content as provided in comment number 65).*	See response to comment number 65.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Linda Birnbaum	67	Summary	My comments are more in general. Overall, EFSA has done an excellent job of reviewing an ever-increasing number of publications re to the issue of PFAS. I applaud their decision to look at 4 significant ones - PFOA, PFOS, PFHxS, and PFNA. Of course, they acknowledge that there are thousands more which is appropriate. The mixture approach which they have taken is pragmatic. They have incorporated certain publications which appeared after the end of their literature, but are missing some key ones.	Thank you very much for your appreciation of the work of the CONTAM Panel
			1. For example, the recent NTP bioassay of PFOA which demonstrated pancreatic cancer at the lowest dose tested should be included, especially as there are human studies showing an association with pancreatic tumors.	1.The draft NTP 2019 study was published after the literature deadline of the current opinion (see also response to comment 48)
			2. There is also a new paper by Blake et al.(Evaluation of Maternal, Embryo, and Placental Effects in CD-1 Mice following Gestational Exposure to Perfluorooctanoic Acid (PFOA) or Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX). Blake BE, Cope HA, Hall SM, Keys RD, Mahler BW, McCord J, Scott B, Stapleton HM, Strynar MJ, Elmore SA, Fenton SE.Environ Health Perspect. 2020 Feb;128(2):27006. doi: 10.1289/EHP6233. Epub 2020 Feb 13. PMID: 32074459) which has relevant information to PFOA .	2. This study was published after the deadline for toxicity related areas.
			3. In addition, there is a recent epidemiology paper by Blake et al. [Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort.Blake BE, Pinney SM, Hines EP, Fenton SE, Ferguson KK.Environ Pollut. 2018 Nov;242(Pt A):894-904. doi: 10.1016/j.envpol.2018.07.042. Epub 2018 Jul 17.PMID: 30373035] of a longitudinal study in Ohio (NOT the C8 cohort).	3.This study was published after the deadline for endpoints judged as non-critical.
			4. Missing old reference re to PFDA: The biochemical toxicity of perfluorodecanoic acid in the mouse is different from that of 2,3,7,8-tetrachlorodibenzo-p-dioxin.Brewster DW, Birnbaum LS.Toxicol Appl Pharmacol. 1989 Jul;99(3):544-54.PMID: 2749739.	4.This paper has been added to the opinion in Appendix D, Table D.1.
			5. Food packaging as a source of PFAS - IN the US, there is data that at least some of the increased levels of PFAS found in African Americans may be associated with higher	5.This study was published after the deadline for exposure and biomonitoring related areas.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			consumption of fast foods. Boronow KE, Brody JG, Schaider LA, Peaslee GF, Havas L, Cohn BA.Serum concentrations of PFASs and exposure-related behaviors in African American and non-Hispanic white women.J Expo Sci Environ Epidemiol. 2019 Mar;29(2):206-217.	
			6. I do NOT understand why the down-rating of the cholesterol response (l. 284-887)when that is a consistent finding in the epidemiological studies (l. 236-37). The decrease in cholesterol in the rat and mouse studies is a function of the peroxisome proliferation, which does NOT occur in people. The unethical studies of Convertino et al (2018) (l. 4687)which exposed dying cancer patients to incredibly high levels of PFOA are not relevant for anything!	6.The reconsideration of the weight of the association between PFAS exposure and increased serum cholesterol is not only due to the absence of similar findings in rodents. A more important reason is the potential shared intestinal reabsorption of PFASs and bile acids, the latter of which can affect serum cholesterol. An explanation of this has now been added to the opinion. We agree that the study by Convertino et al. (2018) has limited relevance in the assessment of causality at much lower exposure.
			7. The state of New Jersey Department of Environmental Quality has done an extensive evaluation of the information on PFNA resulting in the first specific regulation on that congener.	7.Thank you for this information.
			8. There is growing information from the Hokkaido, Japan longitudinal children's study which has found many of the same effects on suppression of the immune system as observed in the Faroe Islands and in the Norwegian birth cohort. Again, overall - this is an excellent document.	8.Thank you for this information.
HBM4EU, the European Human Biomonitoring Initiative	68	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex D of this Technical report.*	Thank you for your support of the approach taken in the opinion and for your contribution to this public consultation and suggestions for further research.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Fidra	69	5. Recommendations	Whilst we welcome the extension of the opinion to species beyond PFOA and PFOS, the scope is nevertheless still extremely narrow given the large number of PFAS on the market. Recognising this to be, in large part, a consequence of limited data availability, we believe the recommendations should go further than simply stating the research needs. Based on the data presented in this opinion, and the recommendations already included, we suggest the addition of the points listed below: 1. Recommendation for regular review and revision as new data becomes available: to gain value from the research recommended in this opinion, the opinion itself must stipulate regular revision to include data in a timely manner, and ensure no undue delay in passing results from publication to protective action. 2. Recommendation that in the absence of sound scientific evidence, proving the human health and environmental safety of PFAS species beyond those directly assessed in this opinion, countries, industries and individual companies should take a precautionary approach, avoiding the unnecessary use of all PFAS wherever feasible. Whilst the interpretation of this recommendation into policy and voluntary industry action is beyond the scope of this assessment, the recommendation remains valid given the likelihood of harm and the absence of evidence to the contrary.	Thank you for your comments. Calls for occurrence data to be submitted to EFSA are made each year. When more data becomes available, then in agreement with risk managers the opinion would be updated. In addition, EFSA can be requested to perform dietary exposure assessments, based on the available occurrence data submitted. The task of the CONTAM Panel was to perform a risk assessment on PFASs in food. The potential consequences and follow-up actions are the remit of risk managers.
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	70	3.2.1.1 Relative contribution from different food groups	It is relevant to quote the study on dietary exposure carried out by by the Istituto Superiore di Sanità the in the Region of Veneto (North Eastern Italy) following the hotspot of pollution of water bodies by industrial release of PFAS, mainly of PFOA. While the levels observed reflect a high-level pollution, the data are relevant to assess the contribution of food categories to overall dietary exposure to PFOA and PFOS (the only PFAS considered). In particular for PFOA, besides drinking water which has represented the main source of pollution, the main contributors to exposure were milk and	Risk assessments of the CONTAM Panel cover the general population and not incidents or hot spots, unless specifically requested by the Commission. However, the derived critical serum levels and TWI will apply also to populations from such areas. The CONTAM Panel is aware that the type of PFASs in such a hot spot may also differ from the ones in the general population and a more specific assessment may be required to deal with that. Also, the relative contribution of food groups and drinking water may be clearly different.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			eggs; in particular eggs could contribute over 45% in consumers of local products. A significant contribution came also from bovine meat in people consuming local products (17% in adolescents). No marked differences were found among age groups, besides the greater importance of milk as source of PFOA in children. The data outline the importance of foods of animal origins and of local feed-to-food chains. The report (in Italian) has been issued in 2019: https://www.regione.veneto.it/web/sanita/informazione-e-comunicazione (https://rdv.app.box.com/s/le1x47b1jgzpfvnzsb446e8ku2fgij1y)	
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	71	3.3.1.4 Transfer from feed to food	Foods of animal origin are the main contributors to dietary exposure, thus suggesting that the role of feeds may be important in food chain contamination. The limited studies presented indicate a significant feed-food bioconcentration in farm mammals but not in fish; this maybe unexpected since fishes are, overall, important contributors to PFAS intake. Moreover, no data are available for chickens, whereas eggs may be a significant source as well. Overall, the transfer from feed to food appears as a gap of knowledge and the need for more data should be included in the Recommendations (see)	This mandate concerns PFASs in food; however, a recommendation for more information on the transfer of PFASs along the food chain is already provided in the opinion. See also reply to comment numbers 74 and 76.
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	72	3.3.5.1.3 Thyroid hormone levels and function	from line 5193 Developmental neurotoxicity is, by far, the most significant effects of thyroid disruption. Whereas "there is insufficient evidence to suggest that exposure to PFASs may adversely affect neurobehavioural, neuropsychiatric and cognitive outcomes." (lines 4240-41), PFOS and PFOA are well recognized as developmental neurotoxicants in rodents (3.3.3.4.1). However, no attempt appears to have been made in order to establish a link between effects in thyroid hormones in pregnancy and/or early post-natal life and neurodevelopmental indexes. This evidence would be important to assess the potential role of thyroid changes in the hazard/risk characterization of PFAS and should be included in the Recommendations (see)	As discussed in the MoA Section (3.3.5.1.3), the effects of PFASs on thyroid hormone levels and the thyroid are unclear. There are indications that the effects on levels of free T4 are caused by an analytical artefact (now added in the MoA section), which might explain why TSH levels do not really increase and the lack of histological changes in the thyroid gland (see e.g. the recent NTP studies). As a result, it is unclear if effects on thyroid hormones could result in neurodevelopmental effects.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	73	3.4.1 Critical effects	Line 5666-71 It is sound and appropriate to identify the immune effects as the critical effects, However it is unclear why the causal association between PFAS exposure and increased cholesterol is now considered uncertain. PPAR alpha is identified as the most relevant PFAS MoA. Activation of PPARalpha is known to impinge on cholesterol metabolism (e.g., cholesterol utilization by mitochondria) as well as on bile acid production; for instance, see for instance this paper on PFNA in mice by Zhang, (Toxicol Sci 2018, DOI: 10.1093/toxsci/kfx237), apparently not quoted in the draft opinion It would be more appropriate to conclude along this line: "a causal association is likely and biologically plausible, even though an alternative explanation exists".	Associations between serum levels of PFASs and cholesterol have been reported in many epidemiological studies. In the absence of a clear confounding mechanism, it was concluded in the 2018 CONTAM Panel Opinion that this association is likely to be causal. The hypothesis that the association may be caused by a common underlying mechanism, i.e. the reabsorption of bile acids and PFASs in the gut, weakens this conclusion and it is recommended to perform more studies to investigate this. This issue is expanded upon further in Section 3.4.1 of the Opinion. Furthermore, effects in animals show the opposite effect. PPARα seems to play a role in certain liver effects but the MoA is unclear and e.g. steatosis seems to evolve from other mechanisms. Similar is true for effects on cholesterol and bile acid synthesis.
			Line 5672-77 PFOS adversely affects the placental development and function in rodents (lines 3015-17); no data exist on possible placental effects by other PFAS. Reduction in foetal weight, albeit not severe, may flag an adverse effect on fetal nutrition with potential adverse health effects later on. It is recommended to replace "the adversity of such a decrease might be questioned" with "the potential longer-term consequences of this decrease are unclear".	The CONTAM Panel agrees and changed the Opinion accordingly.
			Lines 5685-7 It should be specified that no firm conclusions on thyroid effects can be taken in the absence of investigations on the relationship (if any) between thyroid hormone changes in pregnancy and/or infancy and neurodevelopmental impairment.	A recommendation was added to further investigate the effects on thyroid hormone levels and the potential role in neurodevelopmental effects, although at this stage these effects seem to occur at higher doses than the effects on the immune system and mammary gland development.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	74	3.6.1 Uncertainty in exposure estimates	Line 6055 A further uncertainty should be inserted, along the following line:: The lack of robust data on the feed-food carry over in the different farm animal species for the PFAS considered impairs the possibility to model and estimate the transfer and accumulation of different PFAS along food chains. This could lead to either an overestimation or underestimation of the exposure.	The CONTAM Panel used reported occurrence in farm animal species for exposure estimates, and the calculation of transfer from feed to farm animals was not part of the terms of reference. Therefore, the CONTAM Panel is of the view that the lack of basis for estimation of transfer from feed to food is not contributing to uncertainty in the present opinion. However, the CONTAM Panel acknowledges that such data might be helpful for future risk management by the EU Commission.
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	75	3.6.3 Uncertainties in hazard identification and characterisation	3.6.3.2 Epidemiological studies in humans Line 6083 A further uncertainty should be inserted , along the following line: The lack of data on the possible relationship between thyroid hormone changes and neurodevelopmental impairment for the different PFAS considered makes it impossible to assess the role (if any) of thyroid effects in the hazard/risk characterization. This uncertainty may lead to an underestimation of PFAS-related hazards.	The CONTAM Panel does not consider these effects as the most critical and concluded that there is insufficient support for associations between PFOS or PFOA and thyroid disease or changes in thyroid hormones in humans. Furthermore, it recommends to further investigate the decrease in free T4 and the potential impact of the strongly reduced TT4 and TT3 levels at higher dose levels in animals. A recommendation has been added to the Opinion, that the effects on thyroid hormone levels and potential role in neurodevelopmental effects should be further investigated.
Food, Nutrition and Health Unit - Istituto Superiore di Sanità	76	5. Recommendations	Line 6513 Recommended to change as follows "More information is needed on the transfer of PFASs along the food chain, with particular attention to the feed to food transfer" Line 6517 Insert a further recommendation "Studies on the possible relationships between thyroid hormone changes induced by PFASs and adverse effects on neurodevelopment should be carried out".	Based on the uptake of PFASs by plants, it should be considered to not limit studies to food producing animals only. Furthermore, several studies are available on the transfer of PFASs from feed to food, and it should be evaluated which species are actually lacking. See also reply for comment 71.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	77	3.1.4.1 Migration from food contact materials, including non-stick coatings used on cookware	1632-33: 'and therefore have the potential to migrate into food during the cooking process, but studies are inconclusive and show that only small amounts are released into foods'. This sentence surprises us as it seems no longer relevant that extremely small amounts of PFAS, in particular PFOA and PFOS, do have an impact on human health. In fact this is the main subject of this study on the presence of PFASs in food, as we might presume? It might be a good idea to test the so-called PFOA-free and PFOS-free non-stick cookware and see how safe they really are. It occurs that so-called 'regrettable substitutions' create new health hazards.	It was meant that the amounts of PFASs in foods were small when compared to amounts in foods as a result of contamination from other sources e.g. environmental contamination. The wording has been adjusted in this section to make this clear.
Gezinsbond (FlemishFamily Organisation)	78	3.2.1.2 Exposure of breastfed infants	1867-69: 'However, the CONTAM Panel decided that the available data on P95 serum levels are insufficient to estimate the exposure for higher milk levels.' As a family organisation we are particularly worried about the impact of chemicals on infants. We encourage young mothers to breastfeed their babies even up to a year. However this prolonged lactation leads to an accumulation of PFOS and PFOA in the body of the baby. But however infinitely small the amount of these substances, it might have an negative effect in the longer term. Would it be possible to reexamine the exposure risk?	When deriving the tolerable weekly intake (TWI), the CONTAM Panel has taken into account vulnerable groups of the population, including breastfed infants. As stated in Section 3.4.4 '*This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant that are associated with effects on the immune system.*"
			On pag 64 (1912-13) it is observed that intakeof PFAS through consumption of vegetables and fruits also have a considerable impact on infants and toddlers. Is this something we have to be worried about more than the exposure through breastfeeding?	The exposure through consumption of food including vegetables and fruits is relatively small.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	79	3.2.4.1 Exposure through air and dust	As small children crawl on the floor and put anything into their mouth, we think that this kind of exposure ought to get sufficient attention. The same holds for inhalation of air through babies and toddlers that breathe a lot faster than adults. It would be good not to forget that children aren't little adults.	The CONTAM Panel agrees that non-dietary exposure may contribute more to the total exposure for infants and toddlers as compared to other age groups. Text on this has been included in Section 3.2.4.3.
Gezinsbond (FlemishFamily Organisation)	80	3.2.4.2 Dermal exposure	2049-50: 'So far very little is know on the dermal absorption of PFASs'. Here we would encourage more investigation since the skin of young children is much more permeable than that of adults. In this respect the case of BPA is a good example of dermal exposure of an endocrine disruptor.	The CONTAM Panel agrees that there is a need for more studies on dermal absorption, including studies on skin from young children.
Gezinsbond (FlemishFamily Organisation)	81	3.3.3.3 Developmental and reproductive toxicity	As a general observation, we would like to know more about the impact of PFASs on the development of human mammary glands. We suppose that we do not test mice, rats or other rodents in order to conclude that these are not humans. In fact human being are also mammals and so there must be certain links in the mode of action of PFASs on female breasts. Or is this too simply put?	The CONTAM Panel acknowledges a possible human relevance of the impaired mammary gland development after PFOA exposure reported in mice (Section 3.4.1). Effects on the immune system are also observed at low exposure in animals. In contrast to effects on the mammary glands, immune effects have been observed in both animals and humans, and for more PFASs, and therefore constitute a better basis for the risk assessment.
Gezinsbond (FlemishFamily Organisation)	82	3.3.3.5 Immunotoxicity	3868-71: Suppressed immunity by PFOS exposure in mice ought to be investigated to children, from the womb onwards, as exposure starts in utero and continues throughout the breastfeeding episode. It seems to us that this deserves more research inorder to protect new life against infections.	EFSA agrees with the notion that the developing immune system may be vulnerable to the effects of exposure to immunotoxic chemicals. The study by Abraham et al. (2020) is addressing this population group.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	83	3.3.4.1 Fertility and pregnancy outcomes	4081-82: 'In this study (Buck Louis et al. 2018) PFOA was inversely associated with birth weight while for other PFASs no association were observed with birth weight or length.' We are a little surprised to read that PFOA still seems to have an impact on birthweight, while it has been prohibited since 2011 and we recently read that the exposure to PFOA has diminished considerably. So how can we diminish the exposure of pregnant women to PFOA?	This line describes the findings in a particular study, whereas the associations with other PFASs have been shown in more studies. On the other hand, as described in the opinion, there are reasons not to select birth weight as the critical endpoint and more work is needed. However, the CONTAM Panel thinks that the current TWI protects also against these effects, if causal. Measures to reduce the exposure to PFOA and PFASs in general are the responsibility of the risk managers.
Gezinsbond (FlemishFamily Organisation)	84	3.3.4.4 Immune outcomes	4415-42: The recently published study of Abraham et al. about vaccine response to diphtheria, tetanus and haemophilus influenza type B (Hib) refrained by the exposure to PFOA measured in plasma concentrations (and the sum of 4 PFASs (PFHxS, PFOS, PFOA and PFNA) concludes a significant and negative impact on vaccination against tetanus and Hib. The inverse correlation to diphtheriawas only outspoken for PFOA. In these times of corona viruses it seems to us that our youth might better have a strong immunity to confront new pandemics. So we wonder if there will be actions to further diminish the exposure of PFOA and other PFASs in our environment, in particular the exposure through foodstuff.	The CONTAM Panel agrees that effects on the immune system are of concern and identified this as a critical endpoint for PFASs. However, measures to reduce the exposure to PFOA and PFASs in general, are the responsibility of the risk managers.
Gezinsbond (FlemishFamily Organisation)	85	3.3.4.5 Endocrine effects	We are a bit surprised that this opinon concludes that available evidence is inconclusive regarding to possible endocrine disruption caused by PFOA, PFOS and other PFAS. How then can serious deformities of babies born from mothers that had suffered a prolonged exposure to PFOA when working for DuPont. The movie 'Dark Waters' is not just pure fiction in that respect. We as a family organisation put a special interest in the impact of EDCs on the children, since the consecuences might be very serious. Unfortunately even in medical formation very little time is dedicated to the reality of endocrine disruption. So we would appreciate if this part of the scientific opinion of EFSA could get more elaborated than it is now.	The conclusion that evidence is inconclusive means that the available evidence is not strong enough to allow for robust conclusion on health effects. You need consistent findings across different studies to do that in order to account for possible biases that may occur. The health-based guidance value derived on the basis of the immune outcome in this opinion would be protective for health outcomes examined in the C8 studies.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	86	3.3.4.5.2 Male fertility and puberty	See comment before.	See response to comment number 85.
Gezinsbond (FlemishFamily Organisation)	87	3.3.4.5.3 Female fertility, menstrual cycle and puberty	See comment before.	See response to comment number 85.
Gezinsbond (FlemishFamily Organisation)	88	3.3.5.5 Impaired mammary gland development	5633: 'In summary, the mode of action beind the most sensitive PFOA effect, which is a decrease in mammary gland development in animals dosed during gestation and neonatally, is unknown.' We would like to see this conclusion rather as challenge to investigate this impact, since it might reveal cues to the mechanisms of breast cancer of young women and the health impact on girls in their puberty.	The CONTAM Panel agrees that this should be investigated for other PFASs and this is already a recommendation in the opinion, including the relevance for humans. At this stage a possible link with breast cancer has not been shown.
Gezinsbond (FlemishFamily Organisation)	89	3.4.3 Dose-response assessment	5658-59: 'Effects on mammary gland development have thus far not been studied for other PFASs;' As mentioned above, we would like to see more research forthese other PFASs tha.nPFOA,although we realize this might be more a task for the ECHA or the WHO Europe. But we do appreciate that EFSa is using the mixture approach in order to come to conclusions for groups of PFASs.	See comment 88.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	90	3.4.3.1 Effects on the immune system	5854-56: 'It has been shown that during breastfeeding, a substantial part of the PFASs in the mother istransferred to the infant, and as a result,serum levels in the mother but also in the milk decrease over the lactation period.' That is good news for the mother but not for the baby since these 'forever chemicals' will remain for several years in his body. Since we know that endocrine disruption occurs preferentially in certain episodes of the development of the young organism, this is a worrying situation. Once again, we need to give practical advise to breastfeeding mothers in order to limit the exposure of themselves and oftheir babies to PFASs. Can EFSA help us with some practical advises?	The TWI was derived to prevent that exposure of infants could lead to body levels that cause adverse effects. It is also noted that breastfeeding is important for many reasons. Measures to reduce the intake are the remit of the risk managers.
Gezinsbond (FlemishFamily Organisation)	91	3.4.3.2 Mammary gland effects	5933-36: 'Based on the uncertainties on whether these effects on mammary gland development may occur in humans and hte high uncertainties in extrapolation between species, the CONTAM Panel decided to use the effects on vaccination response in humans as the critical endpoint. Nevertheless, this developmental effect is of concern and more studies should be performed to study this effect in humans.' Which acknowledges what we have suggested above...	See response to previous comments.
Gezinsbond (FlemishFamily Organisation)	92	4. Conclusions	6385-86: "there may well be a causal association between PFOS and PFOA and birth weight." We would have liked to see more evidence for this suggestion made by the CONTAM Panel since a reduced birth weight often means that extra health problems might occur in later life of these babies. PS These conclusions give a good and more accessable (for non-specialists) oversight of the EFSA draft scientific opinion.	The inverse association consistently observed for PFOS and PFOA does not appear to translate into clinically relevant outcomes such as low birth weight or small for gestational age.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Gezinsbond (FlemishFamily Organisation)	93	5. Recommendations	6520: 'Studies to characterise the mode of action of immunotoxicity and mammary gland development of PFASs should be performed.' Which of course we applaud...	Noted
Gezinsbond (FlemishFamily Organisation)	94	Appendix A – Levels of PFOS, PFOA, PFNA and PFHxS in different species of fish, arranged according to the LB level for PFOS	no comment ...	Noted
Rolf Soer	95	Abstract & 5.Recommendations	Use 'total PFAS' , instead of four PFASs, for the following reasons: •For the public 'total PFAS' is easier to understand. •The OECD/UNEP paper 'Synthesis paper on paper- and polyfluorinated chemicals' provides a base for defining a class of PFASs , 2013 •Synergistic effects , see EFSA's 'MixTox' guidance, 2019 •It is important to develop HBM indicators in the EU, see: "Development of Policy Human Biomonitoring Indicators for Chemical Exposure in the European Population" with case study PFAS, 2018 •It is common practice for the chemical industry to replace a restricted chemical by its 'brother' which is not restricted. •EU citizens should get the feeling that the EU is protecting them and not the chemical industry.	From a scientific point of view, The CONTAM Panel decided to limit the group TWI to four PFASs, based on structural similarity and half-lives in humans, and their occurrence in human serum. There is not enough basis to include all PFASs in the TWI and further studies should be performed to investigate which PFASs should be included in the TWI. However, it seems likely that also some other PFASs may cause similar effects, some of which show similar persistence in humans. A sentence was added in several parts of the opinion. Furthermore, in the recommendations, also other PFASs are included for further investigation of occurrence in the food chain.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Rolf Soer	96	-	For politicians and EU-citizens it is important to be well-informed on PFAS and to be protected against it. Therefore I have the following question /proposal : Why were only four PFASs chosen? Why is GenX missing? Why not 'total PFAS'? In my country, The Netherlands, Chemours replaced PFOA, after restriction by the Stockholm convention, by GenX or FRD-903. But GenX is probably also an SVHC, see ECHA Annex XV report March 2019: 'Proposal for identification of HFPO-DA and its salts [GenX] as SVHC '. My proposal is to go for 'total PFAS' , instead of four PFASs, for the following reasons: • For the public 'total PFAS' is easier to understand. • The OECD/UNEP paper 'Synthesis paper on paper- and polyfluorinated chemicals' provides a base for defining a class of PFASs , 2013 • Synergistic effects , see EFSA's 'MixTox' guidance, 2019 • It is important to develop HBM indicators in the EU, see: "Development of Policy Human Biomonitoring Indicators for Chemical Exposure in the European Population" with case study PFAS, 2018 • It is common practice for the chemical industry to replace a restricted chemical by its 'brother' which is not restricted. • EU citizens should get the feeling that the EU is protecting them and not the chemical industry.	See previous response to comment number 39. The CONTAM Panel is aware of the potential issues on GenX but this compound was not in the list of PFASs that should be covered by the Opinion. Furthermore, there may be a lack of data to perform a full risk assessment on this PFAS and related compounds.
Rolf Soer	97	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex E of this Technical report (Content as provided in comment number 96).*	See response to comment number 96.

Stakeholder	Comment number	Chapter	Comment	EFSA response
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	98	3.3.4.4.2 Vaccination response	Page 122, line 4418: The COT noted the measuring at different time points, after vaccination, in the Abraham et al (2020) and Grandjean et al (2012) studies (1 and 5 and 7.5 years, respectively) could be an explanation for the difference in potency between the compounds. It was unclear whether there is a correlation between 1, 5 and 7.5 years. For children that were breastfed the impact of the mother's transfer of PFASs will be in the first year. Therefore, the data from the first year may not be as robust as data in the 5-7.5 years age groups. The COT suggested that preference should be given to using the data from the older children. Page 122, line 4439: The levels of PFOS in the plasma were reported to have no relationship with vaccine response in the Abraham study but at similar levels were associated with an effect in the Grandjean study. Page 122, lines 4439-4441: In the analysis of the associations (Appendix K) it was unclear how the data were handled because 80 children had very high levels and 20 children had very low levels of PFOA. These were put together in the analyses and some sort of adjustment was made for the time and number of vaccinations.	In the Abraham et al. (2020) study, only one time point was examined (~1 y) while in the Grandjean study several time points were examined (offering PFASs at age 5 vs antibodies at 5 y and 7.5 y post-booster). Later studies from Grandjean et al. also addressed earlier and later time points. It seems correct that measurements at different ages may explain apparent differences in potency and as shown in the Abraham et al. (2020) study, lactational transfer is dominated by transfer of PFOA, which at later ages contributes less to the overall mixture. The Abraham et al. (2020) study was selected since it provided the lowest HBGV. Furthermore, available studies do not allow derivation of potency factors for this endpoint. Using the Abraham et al. (2020) study was preferred as lactational transfer could be addressed directly.
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	99	3.4.3 Dose-response assessment	Page 154, lines 5853: From the description of the PBPK in appendix M there did not appear to be any information on the evaluation of the EFSA model, although the backbone of the model has been published and reasonably predicts PFAS levels. The modelling used seems to take account of the critical toxicokinetic effects. Compared to the 2018 EFSA opinion on PFOS and PFOA there was not much discussion about the uncertainty around the modelling in this draft opinion. There were a number of caveats about the modelling in the 2018 opinion.	It should be noted that the applied PBPK model is not an EFSA model. The CONTAM Panel added a growth curve in order to study effects in children, and considered the potential impact due to differences between children and adults to be small. Furthermore, an additional exposure route was added, being breastfeeding, which implies not a major change in the model with respect to children. It would affect levels in breastfeeding mothers but this was not the critical age group and therefore not modelled. Information on uncertainties and sensitivity of the model was added to the PBPK Appendix M: model evaluation (uncertainty, sensitivity and model predictions).

Stakeholder	Comment number	Chapter	Comment	EFSA response
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	100	3.4.4. Derivation of a Health Based Guidance Value	Page 158, lines 5942-5944: The COT considered that either the Abraham et al (2020) or the Grandjean et al (2012) study should be used as the critical study as these are the best currently available. The NOAELs from the two studies are not too far apart and there are broad similarities in effects and sensitivity of the two studies However there are also inconsistencies between the studies. Members felt that they were still less than ideal and it would be helpful to have a a more robust point of departure. The mechanism of action is not known and more insights into the mechanism of action are needed.	The CONTAM Panel agrees with this comment. As also suggested by other stakeholders, in the updated opinion, a $BMDL_{10}$ was established from the Abraham et al. (2020) study, as a more robust Reference Point. For the Grandjean study, supportive for the selected critical study, the CONTAM Panel did not obtain enough information to review the performed BMD analysis.
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	101	4. Conclusions	Whilst the COT are unable to suggest an alternative TWI at this time, there will need to be strong caveats explaining the exposure estimates versus TWI relative to exposures and these would need to be considered carefully to avoid miscommunication of the data.	As explained in the Opinion (Sections 3.4.4 and 3.5), the TWI should protect all population groups including infants, because their high PFAS exposure via breastmilk was already taken into consideration when setting the TWI. Therefore, the exposure of infants should not be compared with the TWI.
UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment	102	4.4 Risk Characterisation	Page 173, line 6499: The pathological consequences of the reduction in vaccine response in these children are unknown. It is unknown how this effect relates to the TWI. A one hundred-fold exceedance of the TWI does not necessarily mean that there will be one hundred times greater risk.	The CONTAM Panel decided to base the present PFASs assessment on effects on the immune system. As stated in the opinion a decrease in vaccination response is seen as adverse by the scientific community, as summarised by WHO/IPCS (2012) in the Guidance for immunotoxicity risk assessment for chemicals. A reduction in vaccination response is a measurement of a functional impairment of the immune system. The CONTAM Panel agree that there might not be a linear relationship between exceedance of the TWI and e.g. infection risk, but this is often the case for endpoints, especially when risk factors are applied to derive the HBGV.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Swedish Environmental Protection Agency (SEPA)	103	Abstract	Swedish EPA's comments regarding EFSA's proposed proposed new tolerable weekly intake (TWI) value for perfluoroalkyl substances (PFAS): The Swedish Environmental Protection Agency (Swedish EPA) welcomes a new TWI for PFAS. A new TWI as proposed by EFSA, will in our opinion, lead to improved human health, and will contribute to the Sustainable Development Goals in Agenda 2030. The Swedish EPA's primarily work concerning PFAS regards contaminated sites (soil, sediment, groundwater, surface water), landfills, waste treatment and environmental monitoring. From this perspective, we have read EFSA's draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances in food, and hereby leave our comments. Page 1, line 27: General comments regarding EFSA's proposed TWI for PFAS 8 (ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS): The Swedish EPA sees the need of further clarifying the socio-economic impact assessment regarding the risks to human health related to the presence of perfluoroalkyl substances in food. This is important as the proposed TWI could lead to major impacts on the guideline values for remediation of contaminated soil and ground water, contaminated soil for landfill, the environmetal quality standards and the classification of status for water and laboratory analysis. The Swedish EPA therefore suggests an in-depth analysis concerning this. Guideline values in soil and ground water: The Swedish EPA are committed to giving guidence regarding risk assessment and remediation of sites	The task of the CONTAM Panel was to perform a risk assessment on PFASs. The potential consequences and follow-up actions are the remit of risk managers.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			contaminated by highly fluorinated substances. The Swedish EPA has in collaboration with the Swedish Geotechnical Institute published a joint guidance document where preliminary guideline values for PFOS in soil and ground water are available (Swedish EPA report 6871 published 2019, http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/6800/978-91-620-6871-4/). These preliminary guideline values for PFOS are based on EFSA:s TDI for PFOS from 2008. The intention is to revise our guideline values for PFAS when a new TWI is set. If the proposed TWI is decided, it could lead to Swedish Food Agency decreasing the action thresholde value. As the level of protection of the ground water resource relates to the Swedish Food Agency action thresholde value, this may in turn lead to an adjustment of the guideline value for ground water as a resource. Accordingly, the proposed preliminary guideline value for PFOS in soil for less sensitive land use is governed by the protection of ground water as a natural resource and would be decreased as well. Depending on the magnitude of a future decrease of the action threshold value it is possible that also the proposed preliminary guideline value for sensitive land use would be decreased, which is governed by the protection of soil ecosystem. The Swedish EPA consider there may be large consequences of the proposal. Deposition of contaminated soil: The proposed level of the new TWI would probably affect the amount of landfills needed as a stricter TWI most likely would increase the need for excavation of contaminated soil of larger and more numerous areas contaminated with PFAS. This is an unfortunate consequence as this menas that the already existing problem with contamination being moved around is increased. It also means increased costs, as excavation of contaminated soil is expensive. The Swedish EPA sees a need of new in situ techniques instead	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			of the dig-and-dump-methodology. A lot of research and development is going on in this area, and progression is made constantly. Environmental quality standards and classification of status for water: The level of the proposed TWI may have affected the environmental quality standard for PFOS in water, at least for the annual means in surface water and the limit value for biota, as these values are controlled by the risk for human impact. If the level of the proposed TWI is set, it will also mean less approved waters in the classification of status (Directive 2008/105/EC) leading to need for measures to be taken. Laboratory analysis: The Swedish EPA also wants to highlight that drastically decreased limit values for surface waters and decreased guideline values for soil and ground water could also mean difficulties for the accredited environmental laboratories in offering sufficient low reporting limits.	
Swedish Environmental Protection Agency (SEPA)	104	Summary	Page 8, line 326: General comments regarding EFSA's proposed TWI for PFAS 8 (ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS): The Swedish EPA sees the need of further clarifying the socio-economic impact assessment regarding the risks to human health related to the presence of perfluoroalkyl substances in food. This is important as the proposed TWI could lead to major impacts on the guideline values for remediation of contaminated soil and ground water, contaminated soil for landfill, the environmetal quality standards and the classification of status for water and laboratory analysis. The Swedish EPA therefore suggests an in-depth analysis concerning this. Guideline values in soil and ground water:	See response to comment number 103.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			The Swedish EPA are committed to giving guidence regarding risk assessment and remediation of sites contaminated by highly fluorinated substances. The Swedish EPA has in collaboration with the Swedish Geotechnical Institute published a joint guidance document where preliminary guideline values for PFOS in soil and ground water are available (Swedish EPA report 6871 published 2019, http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/6800/978-91-620-6871-4/). These preliminary guideline values for PFOS are based on EFSA:s TDI for PFOS from 2008. The intention is to revise our guideline values for PFAS when a new TWI is set. If the proposed TWI is decided, it could lead to Swedish Food Agency decreasing the action thresholde value. As the level of protection of the ground water resource relates to the Swedish Food Agency action thresholde value, this may in turn lead to an adjustment of the guideline value for ground water as a resource. Accordingly, the proposed preliminary guideline value for PFOS in soil for less sensitive land use is governed by the protection of ground water as a natural resource and would be decreased as well. Depending on the magnitude of a future decrease of the action threshold value it is possible that also the proposed preliminary guideline value for sensitive land use would be decreased, which is governed by the protection of soil ecosystem. The Swedish EPA consider there may be large consequences of the proposal. Deposition of contaminated soil: The proposed level of the new TWI would probably affect the amount of landfills needed as a stricter TWI most likely would increase the need for excavation of contaminated soil of larger and more numerous areas contaminated with PFAS. This is an unfortunate consequence as this menas that the already existing problem with contamination being moved around is increased. It also means increased costs, as excavation of contaminated soil is expensive. The	

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			Swedish EPA sees a need of new in situ techniques instead of the dig-and-dump-methodology. A lot of research and development is going on in this area, and progression is made constantly. Environmental quality standards and classification of status for water: The level of the proposed TWI may have affected the environmental quality standard for PFOS in water, at least for the annual means in surface water and the limit value for biota, as these values are controlled by the risk for human impact. If the level of the proposed TWI is set, it will also mean less approved waters in the classification of status (Directive 2008/105/EC) leading to need for measures to be taken. Laboratory analysis: The Swedish EPA also wants to highlight that drastically decreased limit values for surface waters and decreased guideline values for soil and ground water could also mean difficulties for the accredited environmental laboratories in offering sufficient low reporting limits.	
Swedish Environmental Protection Agency (SEPA)	105	1.3.4.3 Time trends	Page 22, line 745-746: Read: …Swedish Environmental Agency… Should read: … Swedish Environmental Protection Agency… Page 22, line 745-746: We appreciate that EFSA have used a Swedish EPA report in the supporting material (Swedish EPA report 6513 published 2012, http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/6500/978-91-620-6513-3/). The Swedish EPA would also like to inform EFSA that the Swedish EPA in 2016 reported a Swedish government comission regarding a screening for the presence of hazardous substances, including highly fluorinated substances and pesticides, in surface water and groundwater (Swedish EPA report 6709 published 2016, http://www.naturvardsverket.se/978-91-620-6709-0).	The CONTAM Panel have noted this report but considered that the information on concentrations and temporal trends provided would not change the general conclusions of this section.

Stakeholder	Comment number	Chapter	Comment	EFSA response
Swedish Environmental Protection Agency (SEPA)	106	3.5. Risk characterisation	Page 158, line 5965: General comments regarding EFSA's proposed TWI for PFAS 8 (ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS): The Swedish EPA sees the need of further clarifying the socio-economic impact assessment regarding the risks to human health related to the presence of perfluoroalkyl substances in food. This is important as the proposed TWI could lead to major impacts on the guideline values for remediation of contaminated soil and ground water, contaminated soil for landfill, the environmetal quality standards and the classification of status for water and laboratory analysis. The Swedish EPA therefore suggests an in-depth analysis concerning this. Guideline values in soil and ground water: The Swedish EPA are committed to giving guidence regarding risk assessment and remediation of sites contaminated by highly fluorinated substances. The Swedish EPA has in collaboration with the Swedish Geotechnical Institute published a joint guidance document where preliminary guideline values for PFOS in soil and ground water are available (Swedish EPA report 6871 published 2019, http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/6800/978-91-620-6871-4/). These preliminary guideline values for PFOS are based on EFSA:s TDI for PFOS from 2008. The intention is to revise our guideline values for PFAS when a new TWI is set. If the proposed TWI is decided, it could lead to Swedish Food Agency decreasing the action thresholde value. As the level of protection of the ground water resource relates to the Swedish Food Agency action thresholde value, this may in turn lead to an adjustment of the guideline value for ground water as a resource. Accordingly, the proposed preliminary guideline value for PFOS in soil for less sensitive	See response to comment number 103.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			land use is governed by the protection of ground water as a natural resource and would be decreased as well. Depending on the magnitude of a future decrease of the action threshold value it is possible that also the proposed preliminary guideline value for sensitive land use would be decreased, which is governed by the protection of soil ecosystem. The Swedish EPA consider there may be large consequences of the proposal. Deposition of contaminated soil: The proposed level of the new TWI would probably affect the amount of landfills needed as a stricter TWI most likely would increase the need for excavation of contaminated soil of larger and more numerous areas contaminated with PFAS. This is an unfortunate consequence as this menas that the already existing problem with contamination being moved around is increased. It also means increased costs, as excavation of contaminated soil is expensive. The Swedish EPA sees a need of new in situ techniques instead of the dig-and-dump-methodology. A lot of research and development is going on in this area, and progression is made constantly. Environmental quality standards and classification of status for water: The level of the proposed TWI may have affected the environmental quality standard for PFOS in water, at least for the annual means in surface water and the limit value for biota, as these values are controlled by the risk for human impact. If the level of the proposed TWI is set, it will also mean less approved waters in the classification of status (Directive 2008/105/EC) leading to need for measures to be taken. Laboratory analysis: The Swedish EPA also wants to highlight that drastically decreased limit values for surface waters and decreased guideline values for soil and ground water could also mean	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			difficulties for the accredited environmental laboratories in offering sufficient low reporting limits.	
Swedish Environmental Protection Agency (SEPA)	107	4.3 Critical effects, dose response assessment, derivation of HBGV (conclusion)	Page 172, line 6465: General comments regarding EFSA's proposed TWI for PFAS 8 (ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS): The Swedish EPA sees the need of further clarifying the socio-economic impact assessment regarding the risks to human health related to the presence of perfluoroalkyl substances in food. This is important as the proposed TWI could lead to major impacts on the guideline values for remediation of contaminated soil and ground water, contaminated soil for landfill, the environmetal quality standards and the classification of status for water and laboratory analysis. The Swedish EPA therefore suggests an in-depth analysis concerning this. Guideline values in soil and ground water: The Swedish EPA are committed to giving guidence regarding risk assessment and remediation of sites contaminated by highly fluorinated substances. The Swedish EPA has in collaboration with the Swedish Geotechnical Institute published a joint guidance document where preliminary guideline values for PFOS in soil and ground water are available (Swedish EPA report 6871 published 2019, http://www.naturvardsverket.se/Om-Naturvardsverket/Publikationer/ISBN/6800/978-91-620-6871-4/). These preliminary guideline values for PFOS are based on EFSA:s TDI for PFOS from 2008. The intention is to revise our guideline values for PFAS when a new TWI is set. If the proposed TWI is decided, it could lead to Swedish Food Agency decreasing the action thresholde value. As the level of protection of the ground water resource relates to the Swedish Food Agency action thresholde value, this may	See response to comment number 103.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			in turn lead to an adjustment of the guideline value for ground water as a resource. Accordingly, the proposed preliminary guideline value for PFOS in soil for less sensitive land use is governed by the protection of ground water as a natural resource and would be decreased as well. Depending on the magnitude of a future decrease of the action threshold value it is possible that also the proposed preliminary guideline value for sensitive land use would be decreased, which is governed by the protection of soil ecosystem. The Swedish EPA consider there may be large consequences of the proposal. Deposition of contaminated soil: The proposed level of the new TWI would probably affect the amount of landfills needed as a stricter TWI most likely would increase the need for excavation of contaminated soil of larger and more numerous areas contaminated with PFAS. This is an unfortunate consequence as this menas that the already existing problem with contamination being moved around is increased. It also means increased costs, as excavation of contaminated soil is expensive. The Swedish EPA sees a need of new in situ techniques instead of the dig-and-dump-methodology. A lot of research and development is going on in this area, and progression is made constantly. Environmental quality standards and classification of status for water: The level of the proposed TWI may have affected the environmental quality standard for PFOS in water, at least for the annual means in surface water and the limit value for biota, as these values are controlled by the risk for human impact. If the level of the proposed TWI is set, it will also mean less approved waters in the classification of status (Directive 2008/105/EC) leading to need for measures to be taken. Laboratory analysis:	

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			The Swedish EPA also wants to highlight that drastically decreased limit values for surface waters and decreased guideline values for soil and ground water could also mean difficulties for the accredited environmental laboratories in offering sufficient low reporting limits.	
Norwegian Scientific Committee for Food and Environment	108	Summary	Overall comment The 2020 European Food Safety Authority (EFSA) draft scientific opinion on the risks to human health of per- and polyfluoroalkyl in food is a thorough and comprehensive report. The opinion proposes a group tolerable weekly intake (TWI) limit of 8 ng/kg body weight for the four substances perfluorooctanoic acid (PFOA; CAS 335-67-1), perfluorononanoic acid (PFNA; CAS 375-95-1), perfluorohexane sulfonic acid (PFHxS; CAS 355-46-4), and perfluorohexanesulfonic acid (PFOS; CAS 1763-23-1). EFSA's previous opinion from 2018 established separate TWIs for PFOS (13 ng/kg bw) and PFOA (6 ng/kg bw). The TWI for PFOS was based on increased cholesterol in adults and decreased vaccination responses in children as the critical effects, whereas the TWI for PFOA was based on increased cholesterol. EFSA 2020 reassessed the critical effects and used a new tool for assessing combined exposure to multiple chemicals. Decreased response of the immune system to vaccination was used as the critical human health effect and a group TWI was established for the sum of PFOS, PFOA, PFNA and PFHxS. The new TWI is expected to be protective against other potential health effects, including the increase of cholesterol. The new TWI also protects the most exposed population groups, which include infants, toddlers, and other children. Infant exposure was identified to be the highest during fetal development and breastfeeding. The foods that contribute most to the exposure to these four PFASs were identified as drinking water, fish, fruit, and eggs, as well as products derived from them.	Thank you for your contribution to this public consultation and support of the approach taken in the opinion.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			The Norwegian Scientific Committee on Food and Environment acknowledges the comprehensive and thorough work done by EFSA with this scientific opinion. We agree with your conclusions and find both the decision to use decreased vaccination response as the critical effect in humans, and the use of group TWI based on a multiple exposure approach, to be rational.	
Norwegian Scientific Committee for Food and Environment	109	3.3.4.4.1 Asthma and allergies in children and adults	EFSA concludes that the available evidence is insufficient to suggest that exposure to PFASs are associated with allergy and asthma in children and adults. However, there is increasing evidence for inverse associations between PFASs and atopic dermatitis/eczema. Impinen et al. (Environ Int. 2019;124:462–472) found a reduced risk for atopic dermatitis ever with increasing prenatal exposure to PFUnDA (OR[95%CI]: 0.69[0.55;0.86] per IQR increase) in the MoBa-study (n=1943). In the large Hokkaido Study (n=2062), they found a reduced risk for eczema with increased PFUnDA and PFTrDA in girls (OR[95%CI]: 0.5[0.3;0.81] and 0.39[0.23;0.64] per IQR increase, respectively) and PFTrDA in all participants (0.62[0.45;0.86] per IQR increase) (Okada et al. Environ Int. 2014;65:127–134). In Lowe et al. (Allergy 2019;74(4):812–815), PFUnDA was inversely associated with both atopic and nonatopic dermatitis (multinomial OR[95%CI]: 0.06[0.01;0.76] and 0.05[<0.01;0.18], respectively). In the Spanish INMA cohort, eczema was inversely related to PFOS (RR[95%CI]: 0.86[0.75;0.98] per doubling of PFOS) (Manzano-Salgado et al., Int J Hyg Environ Health. 2019;222(6):945–954). A study published after EFSA's literature search also give evidence for an inverse association (Kvalem et al., Environ Int. 2020;134:105259.). PFNA and PFUnDA levels in 10 year old children were inversely related to atopic dermatitis in	It is true that there are some associations from individual studies that may suggest inverse association. Overall, the current evidence is too inconclusive and the conclusion in the Opinion on asthma and allergy is considered accurate. The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Kvalem et al. (2020) and Lowe et al. (2019) were published after this deadline. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Opinion (Appendix N).

Stakeholder	Comment number	Chapter	Comment	EFSA response
			girls between the age of 10 and 16 years (RR[95%CI]: 0.51[0.35;0.73] and 0.45[0.29;0.69] per IQR increase, respectively). Before adjustment for multiple comparisons, similar associations were also observed for PFDA in girls (0.64[0.42;0.98]) and for PFHxS in all participants and in boys (0.79[0.34;0.99] and 0.71[0.52;0.99] per IQR increase, respectively). As well as between PFHxS and atopic dermatitis in boys at age 16 years (0.59[0.37;0.95] per IQR increase). Even though there are studies showing no effects or a positive association (Averina et al., Environ Int. 2018;114:123–130; Granum et al., J Immunotoxicol. 2013;10(4):373–379; Okada et al., Environ Res. 2012;112:118–125; Smit et al., Allergy 2015;70(6):653–660; Wang et al., Environ Res. 2011;111(6):785–791) and one study showing a positive association (Wen et al., Chemosphere. 2019;231:25–31), a possible inverse relation between PFASs and atopic dermatitis/eczema should not be ignored. Overall, there is increasing evidence for a reduced risk of atopic dermatitis with increasing PFAS levels, especially in girls.	
Norwegian Scientific Committee for Food and Environment	110	3.3.4.4.3 Clinical Infections	The statement that no positive associations between maternal concentrations of PFASs with offspring's later risk of infections were reported in Impinen et al. (Environ Int. 2019;124:462–472) is not correct. Consistent statistically significant associations were reported for lower respiratory tract infections (bronchitis/pneumonia), throat infections and gastric flu/diarrhoea.	Thank you for noting, this has been corrected in the summary to Section 3.3.4.4.3, which is now in line with the drafted description of the study by Impinen et al. (2019).
Norwegian Scientific Committee for Food and Environment	111	3.4. Critical Effects, dose-response assessment and derivation of a health-based guidance value	Page 148 line 5637 and throughout the report: use the term "establish" a HBGV instead of "deriving"	The opinions of the CONTAM Panel use the term 'deriving' in relation to the HBGV.

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Norwegian Scientific Committee for Food and Environment	112	3.4.2 Mixture Approach	We would like the explanation to be clearer under which assumptions and the rationale for choosing a mixture is done, e.g. choosing a component-based model, a dose-addition model etc. We recommend adhering to the steps outlined in the EFSA guidance on mixture. This should also be reflected in the conclusion.	The CONTAM Panel considers that this is clearly stated in the Opinion, i.e. based on occurrence in human serum and included in the available studies on effects on vaccination response. However, a sentence was added that other PFASs may show similar effects, some of which show similar long half-lives in humans.
Norwegian Scientific Committee for Food and Environment	113	3.4.3.1 Effects on the immune system	Page 157. line 5915. Use one third (1/3) of instead of "three-fold lower". The use of -fold lower should be amended through-out the report.	The CONTAM Panel prefers to use the term 3-fold lower, since it is clearer what is meant.
Daikin Chemical Europe GmbH	114	3.4. Critical Effects, dose-response assessment and derivation of a health-based guidance value	It seems that the TDI/TWI may vary, depending on which model to apply. In EFSA's draft report, a group tolerable weekly intake (TWI) of 7 x 1.16 = 8 ng/kg bw per week is identified for the sum of PFOA, PFNA, PFHxS and PFOS. Compared with the (draft) work by Department of Health of US Washington State (Group A Public Water Supplies – Chapter 246-290 WAC Draft Recommended State Action / Levels for Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water: Approach, Methods and Supporting Information, November 2019), EFSA's value is quite low, while Washington State was drafting 'the combination of PFOA, PFOS and PFNA = 3ng/kg/day(=21ng/kg bw/week) and PFHxS = 9.7 ng/kg/day(=68ng/kg bw/week)'. This Washington State's calculation results depends on the model developed by the Minnesota Department of Health (MDH), while EFSA used the model called Physiologically-based pharmacokinetic (PBPK) model. Could you please tell us what EFSA thinks of the MDH model, and how EFSA wants to optimise PBPK models for PFASs (as suggested in the draft report)? Moreover, will there be any efforts by EFSA (or EU) to harmonise the model on the international level in the future?	The CONTAM Panel welcomes the harmonisation of models applied in risk assessment, including PBPK models. This is important to avoid different outcomes based on similar studies. It should be stressed that The CONTAM Panel used data from a recently published study with infants, which may be a major reason for the lower HBGV. In the previous Opinion of 2018, the CONTAM Panel demonstrated that the applied Loccisano model showed similar results as a one-compartment model based on half-life and volume of distribution. In the current Opinion a comparison was added using the MDH-model for PFOA, showing similar outcomes. The draft Opinion includes the recommendation that PBPK models for PFASs should be further optimised.

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			(FYI - this question was askes to the EFSA experts during the workshop on 12 March, and the EFSA experts advised me to submit this question through public consultation.)	
FoodDrinkEurope	115	Summary	SUMMARY Regarding Uncertainties in the food occurrence data Lines 81-142: • After applying exclusion criteria on all food samples received (e.g. samples selected before 2007), the EFSA Panel has finally collected 67839 analytical data collected for 17 PFASs, mainly from Germany, France and Norway. The occurrence data were in general subject to large uncertainties (i.e. different analytical methods with broadly varying LOQ/LOD) and the proportion of results <LOQ was high, with about 90% for all PFASs. The EFSA panel has therefore considered the Lower Bound (LB) exposure estimates to be more realistic than using the Upper-Bound (UB) ones. This suggests however possible underestimations of exposures reported.	The uncertainties in the occurrence data were discussed in the uncertainties Section 3.6.1. The limitations in the analytical methods applied and the large LB-UB differences are also discussed in this section and have been considered in the risk assessment. These uncertainties form the basis of some of the recommendations listed in this opinion. The major reason to trust more the LB exposure, is based on the comparison of intake levels with human serum levels. The UB exposure would lead to much higher serum levels than observed in the various studies.
			• Details of the analytical methods used to determine levels of PFASs and methods performance criteria should be reported.	The data were provided by Member States and analytical methods are only reported briefly in terms of method applied, correction for recovery, and LOQ/LOD.
			• The high portion of left censored data should be considered in the risk assessment. If there is hardly food on the market containing PFAS at detectable levels, the calculated risk for the population should be set in relation to these findings.	The high portion, in combination with the fact that mean and P95 LB exposure exceeds to a large extent the TWI, implies that the sensitivity of the applied methods is in general insufficient. The fact that serum levels indicate the same exceedance, decreases the uncertainty in the assessment.
			• The EFSA Panel acknowledged the lack of information on other non-dietary sources of exposure (e.g. dust ingestion and indoor air inhalation, depending on the site of exposure ('hot-spots') as well as other sources	Agreement noted.

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			containing PFAS) that may significantly contribute to human exposure to PFASs. We agree with this statement.	
			• As a pragmatic approach, the derivation of the TWI was restricted to the mixture of the 4 PFASs only, which represent 46% of the LB exposure to the sum of PFASs. The remaining contribution seems to primarily result from PFASs with short half-lives therefore of probably lower concern. This would need further investigation.	The CONTAM Panel agrees and there is a recommendation that more data on more PFASs should be generated.
			SUMMARY as well as 3.3.5.4 Immunotoxicity Regarding Uncertainties in the human studies and critical effects	
			Lines 311-320, 5558-6517 • The EFSA Panel has considered a recent epidemiological study as pivotal showing an inverse association between serum levels of PFOA and of the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against haemophilus influenzae type b, diphtheria and tetanus in serum sampled from 1-year-old German children, predominantly breastfed. A NOAEC of 31.9 ng/mL at the age of 1 year was derived for the sum of these PFAS from this study that was used to estimate the daily intake by mothers that would result in this serum concentration in breastfed children. Of note, a NOAEC, like a NOAEL, is strongly dependent on the study design (i.e. dose spacing, number of doses) and can be a source of large uncertainty.	The CONTAM Panel agrees and based on the many comments on the NOAEL approach, BMD modelling was further investigated and a BMDL derived that is now the basis for a new TWI. The different approaches are discussed in the Opinion.
			• The EFSA Panel has concluded that effects on the immune system, which were observed at the lowest serum PFAS levels in both animals and humans, is the critical effect. They have considered the findings as robust since in animals they were consistently observed for the two studied PFASs (PFOA, PFOS) and also in humans. A decrease in the vaccination response is interpreted as adverse by WHO/IPCS (2012) but it is difficult to know what the critical effect size considered to be adverse is.	This is a critical issue but the choice of a critical effect size (CES) is in principle more dependent on the uncertainty in the data than the relevance of a numerical decrease in the response. The CONTAM Panel decided to deviate from the default CES of 5% on this data set based on the high variability in the data. There are currently no guidelines on this.

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			• Vaccination response is highly variable in humans. In addition, EFSA has recognized that data on the effects of PFAS other than PFOA and PFOS (such as PFNA and PFHxS) on the immune system is rather limited. The mode of action on immunotoxicity is moreover not fully understood. Lines 382-386 • The EFSA Panel has concluded that more longitudinal epidemiological studies are needed on human endpoints, e.g. prospective vaccination studies covering more varied types of vaccines, different populations, as well as more studies on other immune outcomes including risk of infections. We completely agree with this statement. Moreover, epidemiological studies available to date mostly considered associations between health effects and individual PFASs instead of combined exposures.	Noted Agreement noted.
FoodDrinkEurope	116	3.4.2 Mixture Approach	3.4.2 Mixture Approach Regarding the Assumption on the equal potency of the 4 PFASs Lines 5809-5811 • As a pragmatic approach, the EFSA Panel has decided to restrict the mixture approach to the 4 most abundant PFASs in human serum and in the absence of more specific information assumed equal potencies by default for these four PFASs on immune outcomes. • Studies for deriving potency factors for PFASs are lacking to substantiate this assumption, knowing that the potency is likely related to the chain length of PFASs. Line 153-155 "In humans, the estimated half-lives for short-chain PFASs (such as PFBA, PFBS and PFHxA) were found to range from a few days to approximately one month, whereas for compounds having a long perfluoroalkyl chain length (such as PFOA, PFNA, PFDA, PFHxS or PFOS), it can be several years". PFHxS (perfluorohexane sulfonic acid) has similar half-life to PFAS with >= 8 C atoms (i.e. PFOA, PFNA, PFDA or PFOS), but its chain-length is the same as PFHxA	It is correct and as described in the Opinion, relative potency factors could not be derived. As a default for such instances, the CONTAM Panel applied similar potencies, on a weight base. At this stage there are no studies or techniques that allow to disentangle the specific effects of each PFAS in human studies. The CONTAM Panel can see the point of carbon chain length in PFHxA and PFHxS, but there is currently no clear definition on long- vs short-chain PFASs. The definition would not affect the risk assessment. It is unclear what causes the difference between PFHxA and PFHxS although it should be noted that the sulfonates have a longer overall chain-length than the carboxylates.

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			(perfluorohexanoic acid), which is classified as a short chain PFAS. Consequently, PFHxS should not be reported as a long chain PFAS (as EFSA wrote), but as short-chain PFAS with similar half-life to the long chain ones. And this info is interesting because if we consider shorter chain PFASs, i.e. PFBA and PFBS, they have the same chain length and also similar half-lives.	
			Lines 1127-1137, 1.3.5. Previous risk assessments EFSA has considered that the 4 PFASs share toxicokinetic (TK) properties (in adults- would it be different for infants?), similar accumulation, long half-lives and toxicity effects. It seems that similarities among all 4 chemicals are clearer in terms of TKs properties than in terms of toxicity effects (especially for PFHxS). In addition, other authoritative bodies have set HBGVs, such as the MLR by ATSDR, based on the most critical effects for PFHxS that are different from those reported for PFOA, PFOS, and PFNA.	It is unclear if the TK is different for children. However, children are growing and this affects the apparent half-life. Considering different effects, the most critical effects observed for PFOS and PFOA have insufficiently been compared/studied in animals. Furthermore, separate HBGVs for PFASs would underestimate the potential risk of the mixture.
			• Finally, we are missing a deeper discussion on whether the relative potency should be determined from the applied dose (external dose) or serum/plasma levels (internal dose).	At this stage it is common to base the relative potencies of compounds on external dose, since eventually these factors are also used for product limits. From the recent NTP studies, it is also unclear what would scientifically be the best approach and this is discussed in the Opinion (3.4.2) in connection to Figure 11. In view of the fact that relative potency factors could not be established, the Panel prefers not to expand on this issue.
			Regarding Assumptions used in the PBPK modeling Lines 2317-2356, 5847-5869, 6128-6140 A number of assumptions have been considered in the PBPK modelling such as:	
			• A human PBPK model is applied to translate the critical serum levels into a daily intake (the same as used for PFOS and PFOA by EFSA in 2018), assuming that PFNA and PFHxS behave like PFOA and PFOS, respectively, but EFSA recognizes that data are insufficient for these two PFASs. It could make sense to extend the result on PFOA to PFNA and the one on PFOS to PFHxS based on the presence of the same functional group (i.e. carboxylic and sulfonic respectively). However, it might be explained a bit more in	EFSA decided to base the PBPK modelling in relation to the mixture approach, first of all on similar long half-lives and secondly on similarities in structure. It should also be noted that PFHxS and PFNA contribute only to a small extent to the sum of the 4 PFASs, implying that the uncertainty for the sum is relatively small. However, future improvements in the PBPK model could include a more compound specific approach.

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			detail how this "read-across" has been performed (e.g. if it is based also on physico-chemical properties etc. or if it is solely based on their classification as carboxylic or sulfonic acid).	
			• A breastfeeding duration of 12 months was used. We have the impression that only a small proportion of babies are breastfed for such a long time. A review of median and 95th percentiles of breastfeeding duration might be considered to arrive at more realistic exposure estimates for this population. Other sources of exposure are not included or discussed.	The aim of the modelling is not to mimic the conditions of the study but to derive a TWI that protects most of the population. A breastfeeding period of at least 12 months is advised by WHO and is not uncommon in Europe
			• The age and body weight of pregnant women, and breastfeeding volume of 8oo mL/day Indeed, using a PBPK model, and assuming 12 months of breastfeeding, it was estimated by EFSA 'that the NOAEC in infants corresponds to an intake by the mother of 1.16 ng/kg bw per day for the sum of the four PFASs. Such intake would result in a serum level in the mother at 35 years of age of 12.6 ng/mL. The CONTAM Panel decided to use the daily intake of 1.16 ng/kg bw per day as the starting point and established a group tolerable weekly intake (TWI) of 7 x 1.16 = 8 ng/kg bw per week for the sum of PFOA, PFNA, PFHxS and PFOS. It was decided that no additional uncertainty factors need to be applied'	The CONTAM Panel decided to use the model as published with only minor modifications like a growth curve and adding intake via human milk.
			• Neither enterohepatic cycling nor renal reabsorption was considered in the PBPK modelling. The long elimination half-lives of these PFAS mainly result from their interactions with various transporters involved in the reabsorption processes occurring at hepatic (enterohepatic cycling) and renal (kidney reabsorption) level. We consider the inclusion of these key processes in PBPK modelling as critical.	Enterohepatic cycling is not included in the model but reabsorption in the kidney is an essential part of this model, so this is included. Including enterohepatic cycling would require recalibration of the model, since the parameters for reabsorption in the kidney are fitted to the reported half-lives for PFOA and PFOS in humans. This probably implies that the urinary excretion has to be reduced in favour of faecal excretion. Moreover, the model was validated with serum and not urine data. The CONTAM Panel is aware that if it had used urinary data, a calibration and validation step would have been necessary.

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FoodDrinkEurope	117	4. Conclusions	• The group TWI advocated by EFSA for the 4 PFASs is extremely low, contrary to the values set by other official authoritative bodies so far. The proposed TWI is derived from a human study, the critical effect being related to changes in antibody titres in breastfed infants. We think that the adversity of this effect is highly uncertain and lacks strong substantiation. The EFSA Panel acknowledged a number of assumptions and uncertainties, which are not well-characterised or quantified. Due to these limitations, we wonder if this effect in breastfed infants can be extrapolated to the human population as a whole. Similarly, there are big gaps on the analytical side, including the availability of suitable methods for the different compounds, methods' performance criteria, high proportion of left censored data, missing occurrence data, etc. • To our view, the TWI is overly conservative and speculative, and therefore one could wonder whether the quality and novelty of data is sufficient to refute HBGVs previously derived by expert bodies based on a large amount of animal and human data.	Overall, the CONTAM Panel does not agree that the TWI is overly conservative. The CONTAM Panel concluded that effects on the immune system, which were observed at the lowest serum PFAS levels in both animals and humans, is the critical effect. The findings were considered robust since in animals they were consistently observed for the two studied PFASs (PFOA, PFOS) and also in humans. As stated in the opinion a decrease in vaccination response is seen as adverse by the scientific community, as summarised by WHO/IPCS (2012) in the Guidance for immunotoxicity risk assessment for chemicals. The CONTAM Panel decided not to use additional uncertainty factors as stated in the opinion (see Section 3.4.4 of the Opinion).
Karolinska Institutet / Institute of Environmental Medicine	118	Summary	Row 64 The EC asked EFSA for a scientific evaluation on the risks to human health related to the presence of 27 PFASs in food. The CONTAM panel seem to have made a significant reframing of this task and instead performed a risk assessment for the sum of the four most abundant PFAS (i.e. PFOS, PFOA, PFHxS and PFNA). Two of these where recently evaluated by the same panel, and looking at the very similar outcome, the additional value of this new evaluation is mainly the concept of assessing a group of four chemicals together. This is a pragmatic approach to evaluate the risk of average levels in food and enables the panel to use of epidemiological studies as critical studies (as the epidemiological studies are not able to separate the effects of different PFASs), but the approach holds an important shortcoming for situations where the pattern of exposure deviate from the average. This might be the case in situations where a local source influences the exposure	The CONTAM Panel decided that a compound specific approach for the other PFASs was not feasible, since no data are available for the most critical endpoints. Lack of data also applies to the exposure assessment, which is based on few detected levels. A sentence was added in several parts of the opinion that other PFASs may cause similar effects based on structural similarities, which may be especially relevant for those with (expected) long half-lives. However, at this stage the available data from the critical studies did not allow inclusion of these PFASs in the TWI.

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			and the reference doses (i.e. TWI-values) are very important to perform an initial risk assessment in such cases. Conclusion: There is still a need for a scientific evaluation of the 27 PFASs that was originally asked for by the EC.	
			Row 80 + 187 The CONTAM panel state that relative potencies are "considered not possible" but later concludes that "the most consistent endpoint [increased liver weight, had] clear differences in relative potencies" can be observed (e.g. see row 1138). It is not clear why these potency values are not suitable for the mixture of four PFASs. Conclusion: An alternative approach would be to use the available potency values. This should be tested as a sort of sensitivity analysis.	The CONTAM Panel agrees that applying potency factors based on effects on liver or thyroid hormones in animals could be an interesting approach. However, it is unclear if there are common pathways that lead to the effects on liver, thyroid hormones and the more critical effects on the immune system. At this stage there are no studies in animals that compared the effects of different PFASs on the immune system.
			Row 285 The CONTAM panel now, as compared with 2018, considers the uncertainty regarding causality to be larger when they discuss increased cholesterol levels. However, causality is not only a matter of postulated direct or indirect mechanisms. Conclusion: Criteria for causality, e.g. Bradford Hill, can be used to evaluate if the uncertainty has become larger of smaller. A plausible mechanism is just one of several arguments that need to be commented.	The CONTAM Panel agrees that applying Bradford Hill criteria is a suitable tool. However, it is not only the availability of a plausible mechanism but rather a potential confounding mechanism for cholesterol that led to the decision to select effects on the immune system as the critical effect. Regarding the issue on causality regarding increased cholesterol levels, please see the response to comment 119.
Karolinska Institutet / Institute of Environmental Medicine	119	3.3.4.6.1 Blood lipids	Row 4752 The CONTAM panel now, as compared with 2018, considers the uncertainty regarding causality to be larger when they discuss increased cholesterol levels. However, causality is not only a matter of postulated direct or indirect mechanisms. A new study from a highly exposed population in Sweden confirm the association between PFAS and increased cholesterol. As one of the panel members is a member of the panel the data should have been known (Please include Li et al., 2020 from Environ Health Vol 19, https://link.springer.com/article/10.1186/s12940-020-00588-9)	The CONTAM Panel agrees that causality is not only a matter of postulated direct or indirect mechanisms and that the criteria for causality includes many aspects. EFSA acknowledges that a large number of studies have shown associations between exposure to PFAS and increased serum cholesterol but notes that there is still uncertainty regarding causality due to possible confounding by variability of intestinal reabsorption of PFAS and bile acids. An explanation of this possible source of confounding has now been added to the opinion. We agree that the recent paper by Li et al. (2020), which was published after the deadline argues against such confounding, but uncertainty remains.

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			Conclusion: Criteria for causality, e.g. Bradford Hill, can be used to evaluate if the uncertainty has become larger of smaller. A plausible mechanism is just one of several arguments that need to be addressed if an effects observed in so many different studies are not considered to be causal.	
Karolinska Institutet / Institute of Environmental Medicine	120	3.4.1 Critical effects	Rows 5708 ff Two critical studies on immunological effects (lower antibody titres) were identified. NOAECs of 27 ng/mL and 31,9 ng/mL were identified as a group mean of a quantile below a quantile with a significant effect. The underlying data is presented in Appendix K and L (table K1 and L3). The result shown in K1 seems to be very much depending on the number of sub-groups in the analysis (Q5 or Q10) The data reported in Table L1 refer to larger groups. However, the Q10-analysis show a significant value in group 10. As multiple t-tests are used this might be a false positive value. If the statistics would compensate for multiple comparisons it is not likely that the Q10 would be significant anymore as the p-value is already close to 0.05. In the Q5-analysis the first significant value occurs in the third group, not the fifth. However, the CONTAM panel mark only the fifth group as significant (blue colour) and thereafter use the fourth group as NOAEC. For me, this type of unstable results should be questioned. Conclusion: Although immunological effect seems to be a critical endpoint. The data presented does not seem valid enough as a point of departure for a TWI.	The dichotomous comparisons are post-hoc tests aimed at identifying where a significant decrease occurs. How many groups used to make that comparison (5 or 10) will strongly influence the results, as if too few individuals form the basis of each group, the standard errors are poorly characterised. These results were reported simply to show the underlying uncertainty and how the mean values decrease across groups (leaving all significance aside). The post-hoc analyses are valid in terms of determining NOAECs and it is not considered prone to false positives. This is because the primary analysis reported by the authors clearly shows that there is a significant dose response based on curve fitting (single test).The conclusion on dose response is therefore based on one single test and the resulting p-value and its interpretation are straight forward. The p-values from the post-hoc analyses should be interpreted in that perspective. BMD modelling by the CONTAM Panel, using PROAST also confirms that there is a significant dose response based on fitting either the Hill or exponential models. The CONTAM Panel is of the opinion that this data combined with consistent findings from other studies and animals and humans is valid to derive a reference point for a TWI.
Karolinska Institutet / Institute of Environmental Medicine	121	3.4.4. Derivation of a Health Based Guidance Value	Row 5945 ff Using a PBPK model, and assuming 12 months of breastfeeding, the panel estimated that the NOAEC in infants correspond to an intake by the mother of 1.16 ng/kg/day for the sum of the four PFASs. It was then decided that no additional uncertainty factors should be applied as infants are believed to be the most sensitive population group. However, it seems like the PBPK-model are validated for average humans and from the literature we see that there are considerable individual variations in estimated t½-values. Although we agree that children are a	Model evaluation (uncertainty, sensitivity) has been done and now added to Appendix M according to the WHO/IPCS (2010) guidance. These analyses (sensitivity, uncertainty) show the impact of parameter variability on predicted serum PFOA and PFOS concentrations.

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			sensitive subpopulation there might still be a need to compensate for human-to human kinetic variability. An additional argument for the panel to avoid UFs is that the point of departure refers to a risk factor (lower antibody titres) rather than a disease. We agree that there is no need for an extra UF for severity of the effect. However, this can not be used as an argument for not applying the UF for kinetic differences. Conclusion: To overcome this problem the PBPK-model can be used in a probabilistic mode. A less computer intense approach might be to performed a sensitivity analysis with more conservative parameters, as a sort of sensitivity analysis.	
Karolinska Institutet / Institute of Environmental Medicine	122	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	Rows 6114 ff The CONTAM panel assume equal potency for the four PFASs on a weight basis rather than a molar weight basis. The molecular weight varies quite a lot; 414 (PFOA), 464 (PFNA), 500 (PFOS), 400 (PFHxS). We consider a molar base to be more aligned with the science of toxicology. Most types of molecular initiating events, including the ones that the panel refer to (e.g. PPAR), are interactions on a molar base rather than weight. Weight basis is ok when a mixture is assessed by the hazard index method as the ratios (exposure/RfD) are without unit. However, when the components of a mixture are evaluated as a group with four components the molecular weight should be used. An example: if a mixture consists of mainly PFHxS (MW=400) and the reference data are based on mainly PFOS (MW=500) the margin of exposure will be underestimated by 25%. Conclusion: The risk assessment should be performed on a molar weight basis rather than a weight basis. The TWI can then be "translated" to a weight basis assuming the average composition of the mixture.	The CONTAM Panel agrees that a molar base is scientifically more correct but given the various uncertainties in the assessment and the rounding of the TWI it was decided to use weight based equal potencies. It should also be stressed that relative potencies are often based on order of magnitude (e.g. half log scale).

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Karolinska Institutet / Institute of Environmental Medicine	123	4.2 Hazard Identification and characterisation (Conclusions)	Row 6345 The CONTAM panel report liver tumors (PFOS, PFOA) and Leydig cell tumors (PFOA). In addition to this data, histologic examination has also revealed increased frequences of tumors in the mammary gland in females. The industry has argued that the frequency of mammary fibroadenoma among the treated females was not elevated compared with historical controls. However, when repeated test shows a significant effect it's not correct to use historical controls as an argument to dismiss the control animals of the studies. In addition, pancreatic tumors have also been reported in multiple long-term studies. Conclusion: Tumors in other organs than liver and testis need to be reported as they indicate that PFAS are indeed a multi-site carcinogen in rodents.	Mammary tumour formation has been observed in the past (Sibinski, 1987). The material was re-evaluated critically several-fold, as outlined in Section 3.3.3.7.2 of the EFSA 2018 Opinion. Also, data on pancreatic tumor formation in PFOA-treated animals are equivocal (EFSA CONTAM Panel, 2018). Section 3.3.3.7. of the present Opinion summarises these previous findings.
Karolinska Institutet / Institute of Environmental Medicine	124	Appendix K – Additional information on the study from Abraham et al. (2020)	Table K1 The result shown in K1 seems to be very much depending on the number of sub-groups in the analysis (Q5 vs Q10). The data might be used as supportive evidence, but not as point of departure.	The CONTAM Panel disagrees and refers to the response to your comment number 120 for clarification
Karolinska Institutet / Institute of Environmental Medicine	125	Appendix L – Additional information from the study of Grandjean et al. (2012)	Table L1. The Q10-analysis show a significant value only in group 10. As multiple t-tests are used this might be a false positive value. If the statistics would compensate for multiple comparisons it is not likely that the Q10 would be significant anymore as the p-value is already close to 0.05. In the Q5-analysis the first significant value occurs in the third group, not the fifth. However, the CONTAM panel mark only the fifth group as significant (blue colour) and thereafter use the fourth group as NOAEC. This type of unstable results should be questioned.	Again, the CONTAM Panel refers to the answer to your comment number 120.

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German Federal Institute for Risk Assessment (BfR)	126	Abstract	Line 19 and line 27 (p 1): Readability could be improved if the same units would be used for exposure and TWI (both per day or both per week). Line 30 (p 1): Please add "…..parts of the European population exceed this TWI, which is of concern regarding possible health effects in infants breastfed for a long period of time."	The CONTAM Panel agrees and the main reason was that exposure estimates are normally performed on daily base. However, for clarity this was changed in the abstract. The CONTAM Panel does not agree that the concern is limited to infants. Older children in the Faroe islands showed associations at similar serum levels and a small study with adults suggests potential effects at lower serum levels. It is clear that breastfed infants show the highest serum levels in the general population.
German Federal Institute for Risk Assessment (BfR)	127	Summary	Line 144-145 (p 4): The volume of distribution considerably exceeds plasma volume for all PFAS in all species, see e.g. Han2012. Therefore, plasma contributes only partially to the total volume of distribution. PFAS do not distribute "predominantly in plasma", but rather to plasma and other tissues. Concrete suggestion: Please remove the word "predominantly". It is correct to say that PFAS significantly distribute to plasma, but not that plasma is the main (predominant) reservoir in the body. Line 362 (p 8): Please rephrase and add the text in italics to make the text more informative and precise: "These calculated exceedances of the TWI at LB exposure on the side of the mothers indicate a possible health concern especially for their infants breastfed for a long period of time. Referring to the mode of derivation of the TWI, it is not always feasible to equate an exceedance of a TWI with possible health risks in the respective population group. Health effects are unlikely to occur in the respective population group if the exposure results in serum levels of the sum of PFOA, PFNA, PFHxS and PFOS below 31.9 ng/ml. " Please explain more specifically for which age group a possible health concern results from mean or high LB exposure. Explanation: In the view of BfR, it cannot be assumed that TWI exceedances by a factor of 1.5 during adolescence and / or	The suggested change has been taken into account. The TWI is set to protect all population groups, including breastfed infants. It appears that breastfed infants are the most sensitive group because they show the highest exposure in the general population. However, effects on the immune system may also occur at older age as shown by a small study with humans and studies in adult mice. The following is stated in 3.4.1: *'Although performed with a small group and thus not suitable for deriving a critical serum level, the study by Kielsen et al. (2016) with 12 adults shows that the decrease in vaccination response may also occur at much older age and low serum levels of PFASs (median levels for PFOA, PFHxS and PFOS of, respectively, 1.7, 0.7, 0.4 and 9.5 ng/mL). This is supported by the results from mice studies that were performed with adult animals (see section 3.3.3.5).*' Therefore, it is unclear if a serum level of 31.9 (the NOAEL in the draft sent for consultation) or the BMDL at 17.5 ng/mL would be

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			adulthood result in serum levels higher than 31.9 ng/ml. Thus, these exceedances cannot be considered to be a health concern for these age groups. The serum level of 31.9 ng/ml represents the NOAEC for the most sensitive endpoint in a vulnerable age group. Whether a TWI exceedance by a factor of 4.5 causes serum levels above the NOAEC can and should be answered using the toxicokinetic models.	the Reference Point in adult age groups and more studies are needed. However, exposure below the TWI ensures that serum levels in adults are several fold lower than this value. This is now explained in the Opinion (Section 3.4.4).
German Federal Institute for Risk Assessment (BfR)	128	1.3.1. Chemistry and Synthesis	Line 631, Table1, n:2 flourotelomer alcohols (p 18): We would like to ask why 6:2 FTOH (CAS 647-42-7) was not included in the opinion.	Please see response to comment number 40.
German Federal Institute for Risk Assessment (BfR)	129	1.3.3. Production and use of the substances	Line 651 (p 20): Oil-repellence is also an important feature of PFAS-treated clothing in addition to water- and stain repellence, especially for personal protective equipment and workwear.	This information has now been included in the opinion, in this section.
German Federal Institute for Risk Assessment (BfR)	130	1.3.6. Legislation	page 30: It should be noted that for PFOA in FCM made of plastic, regulation (EU) No 10/2011 is applicable. According to it, the use of PFOA (ammonium salt) as an additive or as polymer production aid is allowed with the specification only to be used in repeated use articles sintered at high temperatures. EU No. 10/2011 regulates further PFAS each with their own specifications. PFNA, PFOS and PFHxS are not allowed for use in the production of plastics food contact materials according to the cited regulation.	This regulation has now been included in the opinion, in this section.

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German Federal Institute for Risk Assessment (BfR)	131	2.2.1. Dietary Exposure assessment	General for this chapter: BfR agrees that all decisions made within the exposure chapters are reasonable and well justified given the challenge of handling an exposure assessment with such a large fraction of left-censored data. In Figures 6 through 9, the draft opinion provides valuable information about the relative proportion of exposure contribution for the individual food groups across the different member states. As the main contributing food groups vary between member states as well as between the specific compound, we would like to request that this information is provided in numerical form, probably best in the form of an additional annex.	This information on relative contribution of food groups to the exposure across surveys has now been added to the Annexes with Occurrence and Exposure data (see Annex A Table A7 and A8). The numerical values behind Figure 6 to 9 have been added to the Annex A (see Table A8). In addition, the numerical values behind Figure 5 (Table A7 in the Annex) have been added.
			Lines 1318ff (p 34, ll). (also concerns ll. 1895ff. p 63,): Please describe the imputation method for assigning occurrence data to food groups with no (or very few) detects in more detail. Currently, the two mentioned passages contain much relevant information, but both have to be read carefully to understand what has been done. For example, the reason why the imputation method was applied – the decision to include as many food groups as possible - is only mentioned in section 3.2.2 but not in section 2.2.1 where the methodology is explained. The same is true for the resulting number of food groups in the exposure assessment. BfR agrees that this is a well-reasoned approach to calculate the overall dietary exposure to PFAS. However, the description of this very important decision including the rationale behind it and impacts should be given more room. This is even more important since it was handled differently than in the 2018 opinion. Additionally, information should be given about how the imputation method impacts each food group.	The CONTAM Panel agrees to include more specific information in Section 2.2.1. on the imputation method used and information has been added accordingly.
			Lines 1334ff (p 35): Have the occurrence samples been analysed for co-occurrence of the four compounds? If yes, this could be given some appraisal in the section regarding the	No this was not analysed. This would certainly be interesting, although it would not affect the exposure assessment for substances with long half-lives. The exposure was based on the

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			occurrence data. In any case, a possible correlation of the occurrence of the compounds can have impact on the summation approach and should be mentioned either in this chapter and/or later in the uncertainty section. Lines 1343-1344 (p 35d): How is the LB and UB-approach applied for the combined exposure of the four PFAS, e.g. when more than one substance has a value below LOD/LOQ or when there is a mix of values below LOD/ LOQ and quantified values? Line 1322 ff (p 34) Correction: We believe that this should be FoodEx 2 level 1 and not FoodEx1.	mean levels of each PFAS. These were subsequently summed for the 4 PFASs As explained, this was not applied in this way. Values for LB and UB were summed for the 4 PFASs, so in the case of UB it is possible that quantified values were summed with LOQs values. For LB only quantified values are summed as results below LOQ are assigned a LB value of zero. The correct text should be FoodEx Level 1.
German Federal Institute for Risk Assessment (BfR)	132	3.1.2. Analytical results	Lines 1538-1543 (p 42): Detection rates of PFAS are lower in vegetables compared to fruits. Is this because the tested vegetables were primarily roots? Which kinds of vegetables have been included? Or is this due to the fact that plants producing fruits are usually perennials and can accumulate more PFAS over the years?	The CONTAM Panel noticed that the detection rates in vegetables analysed in the Perfood project are lower than for fruits in the same project, however there is not sufficient information available to draw conclusions on differences between fruits and vegetables The dataset used for the exposure assessment includes several types of vegetables, including brassica, bulb and fruiting vegetables, fungi, leafy, legume, root and stem vegetables (fresh), sugar plants and vegetable products.
German Federal Institute for Risk Assessment (BfR)	133	3.1.4.1 Migration from food contact materials, including non-stick coatings used on cookware	page 46: It should be noted that for PFOA in FCM made of plastic, regulation (EU) No 10/2011 is applicable. According to it, the use of PFOA (ammonium salt) as an additive or as polymer production aid is allowed with the specification only to be used in repeated use articles sintered at high temperatures. EU No. 10/2011 regulates further PFAS each with their own specifications. PFNA, PFOS and PFHxS are not allowed for use in the production of plastics food contact materials according to the cited regulation. Lines 1667-1670 (p 47) The current consideration of FCM as a potential contribution to PFAS exposure is incomplete. In 2019, RIVM published a comprehensive overview, which found that more data is needed, especially on the migration	This regulation has been included in Section 1.3.6 of the Opinion (see response to comment number 130). Please refer to the response to Comment 43 above which also highlighted the reports, and that our literature search for this topic focussed on articles published in peer reviewed journals between 01 2007 and 09 2018.

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			of contaminants and contaminants - including PFAS - from different types of FCMs into food. DOI: 10.21945/rivm-2018-0181 Most literature is on PFAS content but not on migration into food. Below are some publications that contribute to the current state of knowledge: doi: 10.6027/NA2015-911 and 10.6027/TN2017-543 report amounts of extractable PFASs (PFCAs, PFSAs, FTOHs) in Nordic consumer products including FCM. two further studies (doi: 10.1080/19440049.2013.789556 and 10.1021/acs.est.5b03806) published data on the occurrence and migration of PFAS with regard to FCM	
			Line 1639 (p 46) In the study of (Zabaleta et al., 2017), no migration testing was performed, but rather an extraction with methanol under ultra sonification.	It was stated that the study did not measure migration, but the wording has been adjusted to further clarify.
			Lines 1640 ff (p 46) In the study of Schaider et al. (2017), a total fluorine analysis was performed to detect whether PFAS have been used in the production of the respective paper sample. A comparable method was also used by the Danish EPA, but in the last years, it became obvious that due to background presence of fluorine (e.g. from inorganic fluorine compounds) in the paper, it is not possible to reliably and sensitively distinguish between papers containing PFAS and background fluorine content. Hence, this methodology will no longer be used for the sensitive detection of PFAS, but will presumably be replaced by methods, which detect specific substances.	It is stated in the Opinion that this study did not measure concentrations in food as a result of migration, and the CONTAM Panel agrees that other sources of fluorine in food may have an impact on this approach.
			Lines 1651 ff (p 47) It should be noted that (Choi et al., 2018) tested repeated use articles in more than one consecutive test (as stated in reg. (EU) No 10/2011, where the third consecutive test is used for evaluation of substance release). For frying pans, pots and other coated repeated use articles, with PFAS release in the first migration test, they found no PFAS	This point has been clarified in the text.

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			release in the following tests. This means that PFAS exposure from these articles should only occur after the first use and not on a daily basis. Line 1670 (p 47) To what extent do FCMs contribute to daily PFAS exposure, e.g. compared to food? Is it likely, that contributions from packaging and processing of food are already included in the exposure estimation? Or should they be added? Although the data from the literature do not provide a comprehensive picture of the different PFAS-containing FCM and their impact on human exposure, it seems quite clear that PFAS-release from FCMs depends highly on the specific FCM. Human exposure due to the release of PFAS from PTFE non-stick cookware coatings seems to be negligible. Only a very small impact on overall PFAS-exposure is expected from the occasional use of PFAS-equipped paper packaging. A significant exposure might result from the very frequent use of PFAS-equipped paper packaging in contact with hot food (e.g. microwave popcorn, fast food).	It was stated earlier in the section that exposure as a result of migration from FCMs is small compared to other sources, but the wording in both places has been modified to make this clearer. There is a large uncertainty in estimates relating to this source of exposure, and whether certain population sub-groups may be disproportionately affected. This supports the recommendation that '*More studies on the effect of cooking and food processing, in particular in relation to transfer to food from food contact materials that contain PFASs, are needed*.
German Federal Institute for Risk Assessment (BfR)	134	3.2. Dietary exposure assessment	General for this chapter: BfR agrees that all decisions made within the exposure chapters are reasonable and well justified given the challenge of handling an exposure assessment with such a large fraction of left-censored data. In Figures 6 through 9, the draft opinion provides valuable information about the relative proportion of exposure contribution for the individual food groups across the different member states. As the main contributing food groups vary between member states as well as between the specific compound, we would like to request that this information is provided in numerical form, probably best in the form of an additional annex.	The numerical information has been added, please see response given for comment number 131.

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German Federal Institute for Risk Assessment (BfR)	135	3.2.1.2 Exposure of breastfed infants	Lines 1854 – 1869 (p 63) Assumptions for breastfed infants in this chapter (infants below 6 months of age: average daily milk consumption 800 mL, high consumption 1200 ml, bw 6.1 kg) are not consistent with the assumptions taken into account in the modelling of the exposure of the breastfed infant (p. 154, lines 5866-5877: breastfeeding period of 12 months, intake 800 ml; p. 158, line 5973/74: intake of 800 mL human milk, average body weight of 6.1 kg during the first year) as well as with the Berkeley Madonna code (Appendix M, Lines 10865-10889 and 10987-11007 Variable Milkconsumption=0.8 L). Please use consistent assumptions along the opinion. For instance, it should be clearly stated that the amount 800 mL was used for modelling throughout the opinion (while the high consumption of 1200 mL was used exclusively in 3.2.1.2 to calculate an example of high daily PFOS and PFOA intake based on the assumptions of EFSA NDA Panel).	The CONTAM Panel routinely calculates the exposure of infants via human milk and for this applies an average and high intake of 800 and 1,200 mL per day. This is only meant to give an impression of the exposure as compared to older age groups. The 1,200 mL per day is now included in the sensitivity analysis of the PBPK model, among other parameters. It was decided not to use this higher intake of milk for deriving the TWI regarding the many uncertainties in the assessment and the nature of the endpoint. A sentence was added to this section that more details on the exposure of infants and the interpretation of the estimated exposure can be found in other sections of the Opinion.
German Federal Institute for Risk Assessment (BfR)	136	3.3.1.2 Humans (TK)	Line 2152 (p 69) Please add 8:2 before FTOH	Added
			Line 2230 (p 70) The half-lives of PFOA and PFOS are, according to Table 15, 1.2-8.5 a and 1.9-18 a Could you please comment why the figures in the text differ from the ones in Table 15?	The apparent discrepancy between the text and Table 15 regarding the half-lives is due to the fact that the range given in Table 15 is expressed as geometric mean (as explicitly mentioned), whereas in the text, for comparison purposes with other PFASs (and referring to EFSA and ATSDR reports), the values are reported as arithmetic mean, as now mentioned in the revised version.
			Line 2270 (p 71) In order to calculate the maternal-infant transfer via breast milk, EFSA used the ratio of the concentrations in milk versus plasma. For PFOA a range of 0.03-0.12 is given. According to Appendix M, for the PBPK modelling approach (page 445, line 10833) 0.03 was used. Could you please explain why you chose to use the lowest number only? Would it be possible to include also the value of 0.12 in the PBPK modelling (fig. 14)? (An analogous argument holds true for PFOS)	Model evaluation (uncertainty, sensitivity) has been done and now added to Appendix M according the WHO/IPCS (2010) guidance. It appears that such variations in maternal-infant transfer via breast milk have a medium influence on the serum concentration at the age of 1 year estimated with the PBPK model.

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			Line 2281 (p 71) The decrease in breast milk concentrations of PFOA ranges between 1.3-7.7%. For the PBPK modelling (page 447, line 10931) the number 0.077 was used for the decline only. Could you please explain why you chose to use this value? Would it be possible to include the value of 0.013, too? (An analogous argument holds true for PFOS)	
			Lines 2229/2283 (p 70/71) There are new data available on PFHxA that estimates its half-life in serum a 5.1 days. Luz A.L., Anderson J.K., Goodrum P., and Durda J. (2019): Perfluorohexanoic acid toxicity, part I: Development of a chronic human health toxicity value for use in risk assessment. Regul Toxicol Pharmacol 103, 41-55. DOI: 10.1016/j.yrtph.2019.01.019, therein, another study is cited: Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., De Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated Environ. Assess. Manag. 7 (4), 513–541. (https://www.daikinchemicals.com/library/pb_common/pdf/sustainability/PFHxA/PK_003.pdf)	Luz et al. (2019) published a paper dealing with PFHxA toxicity in which they refer to a half-life of 5.1 days, based on Buck et al. 2017 data (and not Buck et al et al., 2011 as mentioned by BfR). These data, although presented in 2017 at an ACS meeting, have not yet been fully published in a scientific journal. Furthermore, in the discussion of the article by Luz et al. (2019) the half-life of PFHxA was discussed as approximately 32 days rather than 5.1 days. For these reasons, the CONTAM Panel decided not to mention the value of 5.1 days as the half-life of PFHxA, considering these data as insufficiently substantiated and reported.
			Another new study (Hethey et al., not yet published) is available and cited in the Annex XV report for the restriction of PFHxA and related substances (https://echa.europa.eu/de/registry-of-restriction-intentions/-/dislist/details/0b0236e18323a25d, Appendix B.5.1), which estimates the half-life as 7.2 days.	The study from Hethey et al. has not been published yet and cannot be taken into consideration.
			Line 2269 (p 71) Typo: one of the two commas should be removedPlease add 8:2 before FTOH	Corrected

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German Federal Institute for Risk Assessment (BfR)	137	3.3.2.6 Factors that may have an impact on the internal dose of PFASs	Line 1592 (p 82) There is also a recent study showing PFAS levels in different human fetal/embryonic tissues (Mamsen et al., 2019)	The CONTAM Panel applied a deadline for literature to be included, since it is not pragmatic to include all literature up to the publication date of the Opinion. Literature was screened and only papers considered relevant for the assessment were included. For some critical endpoints the deadlines were moved to a later date regarding the relevance of some of the papers for the assessment, and for effects on the immune system even a draft paper was taken into consideration. This has been clarified in the Oopinion (Appendix N).
German Federal Institute for Risk Assessment (BfR)	138	3.3.2.7 Relative contribution and correlations between PFASs	Line 2650 (p 84) Please specify what is meant by "medians of medians". Is it "The contribution of various PFASs based on the median of the median serum levels in biomonitoring studies in Europe"? If so, please add an appropriate explanation here for completeness and citeability. Line 2635 (p 83) Correction: It is Figure 10, not Figure 4	The CONTAM Panel agrees and has added this information accordingly. The CONTAM Panel agrees and has corrected this.
German Federal Institute for Risk Assessment (BfR)	139	Effects following repeated exposure 3.3.3.2.3 Other PFASs (Effects following repeated exposure)	Line 2970 (p 90) 6:2 FTOH (CAS 647-42-7) should be included in the opinion. For this substance, a variety of in vivo animal toxicological studies is available pointing to adverse effects on teeth (mottled teeth and delamination of the low incisor tip surface which might have serious consequences for the stability of the teeth).	See the response to comment number 40.

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German Federal Institute for Risk Assessment (BfR)	140	3.3.3.3 Developmental and reproductive toxicity	Lines 3089 ff (p 92 f) For the study of White et al., 2011, it should be noted that the chosen endpoint of pathological score (0-4) of the mammary gland shows high individual variation (e.g. compare Table 1, F0 dams on PND 22 in control + 5 ppb PFOA (score 3.4) with Table 3, F1 dams in the same group at the same time point (score 2.2); or compare Table 2, F1 control pups at PND 22 – 63 (score 3.8) with Table 4, F2 pups in the same group and in other groups (1 mg/kg or 5 mg/kg only for the F0 dams) with comparable PFOA blood concentrations (see Table 5) -> score in a range of 2.0 – 3.5 Hence, the occasionally seen statistically significant differences of the 5 ppb group compared to the control group should be treated with caution, since the respective parameter (pathological score) is highly variable over comparable groups and controls. The LOAEL of the study is rather the 1 mg/kg bw/day group and not the 5 ppb group.	The LOAEL is based on effects reported in F1 (lines 3096-3098): *'Mammary gland development scores were significantly decreased in all treated F1 pup groups on PND 22, 42 and 63, indicating that at the time when F1 females became pregnant, the mammary glands were not fully developed.'* There was a statistically significant decrease already in the lowest dose group (5 µg/L), and there seems to be a dose-related effect on PND22 (Table 2). These effects are, in the CONTAM Panel's view, not as stated by BfR '*occasionally seen statistically significant differences',* since similar is observed also on PND42 and PND63. The CONTAM Panel confirms that the study LOAEL is 5 µg/L of PFOA. Comparing developmental scores in control groups in F1 and F2, does indicate variations for unknown reasons, and only occasional and inconsistent significant effects are observed. However, the lack of effects in F2 is not the basis for the LOAEL. Increased scores in P0 dams (Table 1) versus control indicates a delay in involution in P0. This effect is not easy to interpret on PND 22 in F1 dams (Table 3), with significantly decreased score (increased involution) in the 1 mg/kg bw + 5 µg/L in drinking water group, and increased score (delayed involution) in the 5 mg/kg bw group. This is however not the basis for the LOAEL as explained above.
German Federal Institute for Risk Assessment (BfR)	141	3.3.3.3.3 PFCAs (Developmental and reproductive toxicity)	Line 3214 (p 95) The NTP study also reported findings on thyroid hormones, which should be added.	These findings were reported in the table E.1 of Appendix E in the opinion.

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German Federal Institute for Risk Assessment (BfR)	142	3.3.3.3.4 PFSAs (Developmental and reproductive toxicity)	Line 3456 (p 99) The "endocrine disruptor mix" should be explained. Which substances are included in this mix? Line 3459 (p 100) Does "profound" mean statistically significant?	In their paper Ramhoj et al. (2018) investigated PFHxS dose dependently as a single compound, an endocrine disruptor mix and PFHxS in combination with the endocrine disruptor mix. The CONTAM Panel used only the data obtained following exposure to PFHxS. The endocrine disruptor mix therefore needs no further explanation. The text in the opinion reads 'pronounced' and not 'profound'. The CONTAM panel agrees, that this is not sufficiently specific. This has been amended to 'pronounced, statistically significant at doses from 5 mg/kg bw per day of T4 and higher'.
German Federal Institute for Risk Assessment (BfR)	143	3.3.3.5 Immunotoxicity	Whole chapter: Please refer also to the NTP metastudy on immunotoxicity of PFOS and PFOA: https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf. Line 3646 (p 103) Typo: should be "decrease" instead of "decreased"	This meta-study was referred to in the 2018 Opinion (EFSA CONTAM Panel, 2018) (Section 1.3.6 on Previous Risk Assessments), it was not considered necessary to include it in this opinion.
German Federal Institute for Risk Assessment (BfR)	144	3.3.4.4 Immune outcomes	Whole chapter: Please refer also to the NTP metastudy on immunotoxicity of PFOS and PFOA: https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf.	See response to comment number143.
German Federal Institute for Risk Assessment (BfR)	145	3.3.5.2.1 Hepatocellular cytotoxicity, steatosis and increased liver transaminases in serum (MoA)	Lines 5457-8 (p 144) The description mentioned IL10 as a pro-inflammatory cytokine. However, IL10 is an anti-inflammatory cytokine and the cited reference (Fang et al. 2012a) describes an increase of the anti-inflammatory cytokine IL10. Please correct.	The CONTAM Panel agrees that IL10 is not a pro-inflammatory cytokine and the respective text was corrected.

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German Federal Institute for Risk Assessment (BfR)	146	3.4.1 Critical effects	Line 5700 (p 149) The assessment is on the serum levels for the sum of four PFASs, i.e. PFOA, PFNA, PFHxS and PFOS. At present, these are the PFAS that contribute most to human serum levels. Please explain why the assessment is not done on the six PFAS that contribute most to the levels (PFOA, PFNA, PFHxA, PFOS, PFDA, PFUnDA) and why PFHpS is not included. Why were PFDA and PFunDA not included in the risk characterisation/ the TWI derivation? As long-chain PFAS, they have long serum half-lives and the median of the sum of PFDA and PFUnDA in adults is equal to the median of PFNA. As we have to deal with occurrence of PFAS other than those included in the proposed TWI in food, an explanation why long-chain PFASs were excluded from the EFSA opinion would be helpful for our risk communication strategy. It would also be acceptable if the exclusion of long-chain PFAS was just "for practical reasons" or "used as a practical approach" if that is indeed the case for this EFSA opinion.	The CONTAM Panel decided to include only those PFASs that were measured in the serum of infants and children in the critical studies. Since there are no data on other PFASs for the critical effects, no specific risk assessment was performed. However, a statement was added that also other PFASs may cause effects on the immune system based on structural similarities and especially those with a long half-life in humans may be relevant.
German Federal Institute for Risk Assessment (BfR)	147	3.4.2 Mixture Approach	Line 5754 (p 150) The selection of four substances does not cover the dynamics and future trend in the use of PFAS sufficiently (e.g. decrease of the occurrence of PFOA and PFOS and concomitant increase in other PFAS). Shorter half-lives do not represent a convincing and sufficient argument to exclude certain substances from the evaluation.	This is correct, and as stated in the next line The CONTAM Panel '*acknowledges that more data are needed on other PFASs in the future*'. It should be noted that for PFASs with short half-lives, much higher exposure seems to be required to obtain similar serum levels as for the PFASs covered by the Opinion, unless they would be much more potent. As illustrated in Figure 5 in the Opinion, approximately half of the dietary exposure comes from other PFASs than PFOA, PFNA, PFHxS and PFOS, and still these 4 constitute more than 85% of the median serum values in children and adults. Furthermore, there are no specific studies on effects on vaccination response from other PFASs.
			Line 5766 (p 151) Discussion on grouping based on toxicological effects and discussion on potency deserves more attention.	The CONTAM Panel acknowledges that this is a pragmatic approach (as stated in Section 3.4.2) and better than assuming no mixture effects. However, more discussion would not make this issue much clearer since both MoA and specific comparative data on immune effects are lacking.

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			Lines 5800-5808 (p 152) Here it could also be mentioned that the LOAEC of 40.5 ng/mL (corresponding to ~ 0.08 - 0.1 µM serum concentration, or even half of that in whole blood) is two orders of magnitude below the observed activation of human PPARs as stated on p137 (C20max >10 µM). This contradicts a participation of PPARs in the immunotoxic MoA.	As stated in 3.3.5.4, there is no support for a role of PPARα in the decreased immune response, based on studies with KO animals. This is now discussed in the MoA section.
			Table 21 (p 153) Shouldn't the Wyeth/Serum freeT4/F- cell be blue (1.5->increase)?	Yes, thank you for noting, the cell colour in Table 21 was changed.
German Federal Institute for Risk Assessment (BfR)	148	3.4.3 Dose-response assessment	Lines 5822-5918 (p 154) The TWI is calculated based on a proportion of 28% PFOA+PFNA and 72% PFOS+PFHxS for both background exposure and serum levels at 35 years of age. What would happen if the proportion of PFOA were higher, but we still respect the TWI of 8 ng/kgBW/wk? To explore this, we look at the case that the TWI is exhausted exclusively from PFOA, such that mothers have a plasma concentration of 12.6 ng/ml and the children from 1 year of age consume 8 ng/kgBW/wk of PFOA afterwards. The resulting plasma concentration can be seen in Fig 1 of the present document (see uploaded file) to exceed the NOAEC of 31.9 ng/ml at the critical time between 0 and 1 years of age. We would like to see this explained in the uncertainties. It is possible for populations exposed from e.g. drinking water with high PFOA (but still under the TWI for food and drinking water together) to achieve serum concentrations such that their breast-fed children exceed the NOAEC of 31.9 ng/ml.	Since PFOA shows a higher transfer to milk than PFOS, infants from mothers with a body burden of primarily PFOA would indeed achieve serum levels higher than the $BMDL_{10}$ of 17.5 ng/mL now used, instead of the NOAEC. The estimated serum level would be 30.3 ng/mL in the case of exposure to 0.63 ng/kg bw per day to only PFOA. To prevent this, the mothers should not exceed a weekly intake of 2.6 instead of 4.4 ng/kg bw. However, the CONTAM Panel considers it unlikely that people are only exposed to PFOA, unless there is a specific hotspot which would result in in much higher exposure and serum levels.
			Lines 5860-5863 (p 154) Exposure of children at age 1 to 10 was assumed to be 0.33 ng/kg bw per day for PFOA and 0.83 ng/kg bw per day for PFOS (1.16 ng/kg bw per day for all four PFAS) in the model described in lines 5860 to 5863 (Figures 12 and 13).	In lines 5860-5863, the exposure of the mother is described, not that of the children. It is true that for the mothers, the double intake during childhood was not included in the graphs for the mothers, since it would cause a sudden two-fold lower intake at

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			However, the exposure for this age group was assumed to be two times higher (0.66 ng/kg bw per day for PFOA and 1.66 ng/kg bw per day for PFOS) in lines 5878 to 5880 (figures 14 and 15) according to the estimated exposure in chapter 3.4.3. Please use consistent assumptions for the two modelling approaches. This would also clarify whether or not persons exposed to levels twice the TWI at age 1 to 10 would end up with blood levels higher than 12.6 ng/mL at age 35.	the switch from child to adolescent age. However, a two-fold higher exposure at young age does not affect the body burden and serum level at child-bearing age, due to increase in body weight and considering the half-lives of the compounds.
German Federal Institute for Risk Assessment (BfR)	149	3.4.3.1 Effects on the immune system	Line 5858 ff (p 154) …" the serum level of 31.9 ng/mL was the sum of levels of PFOA, PFNA, PFHxS and PFOS of 15.1, 0.5, 2.1 and 14.2 ng/mL". Here, a reference is missing, as these data are not in the Arch Toxicol paper: (Abraham, 2020, personal communication)	This has been updated based on the published data for breastfed infants.
			Line 5858 ff p 154 PBPK modelling: the model needs data to predict the development of body weight during the first year of life. The data are based on a French study (EAT for French total Diet Study) which "includes 4,078 subjects with age between 3 and 60 years, and 703 subjects of less than 3 years. The reported data (weight, age) from this study are used to build an equation describing the increase in weight according to age." (Line 10776, equation line 10916). Such a third degree polynomial body weight equation, valid for the complete lifetime, is not expected to adequately describe the weight development during the first year of life (see Figure 2 in uploaded file). Most importantly, this discrepancy in body weight has consequences for the outcome (TWI). The body weight at the age of one year is 8.06 kg only (EFSA equation, Fig. 2 of the present document), whereas boys have a mean bodyweight of 9.65 kg and girls of 8.95 kg (mean 9.3 kg). The higher body weight at the age of one year results in lower PFAS concentration if the intake is not changed. Using the model with the WHO mean data (boys and girls), the maternal concentration before pregnancy changes to 4.05 ng/mL (PFOA) and 10.65 ng/mL (PFOS), from 3.4 and 9.2	For body weight, the CONTAM Panel explored the body weight influence with different growth curves from WHO until the age of 5 years (now presented in Appendix M): Depending of the growth curve used (WHO P50 girls, P50 boys, P95, PO5), the calculated PFOA /PFOS serum concentrations with PBPK at 1 year is impacted but remains acceptable, since all the difference between all predictions are less than a factor 2 (WHO/IPCS, 2010).

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			ng/mL, respectively, in order to reach levels of 15.6 ng/mL (PFOA) and 16.3 ng/mL (PFOS) in the one-year-old children, respectively. To reach the maternal levels mentioned (females with the age of 35 years), the daily intake has to be 0.38 ng/kg bw (PFOA) and 0.96 ng/kg bw (PFOS), instead of 0.33 and 0.83 ng/kg bw, respectively. Therefore, the TDI for the PFAS sum would change to 1.34 ng/kg bw, changing the TWI to 9.4 ng/kg bw. Lines 5878-5880 (p 155) Exposure of children at age 1 to 10 was assumed to be 0.33 ng/kg bw per day for PFOA and 0.83 ng/kg bw per day for PFOS (1.16 ng/kg bw per day for all four PFAS) in the model described in lines 5860 to 5863 (Figures 12 and 13). However, the exposure for this age group was assumed to be two times higher (0.66 ng/kg bw per day for PFOA and 1.66 ng/kg bw per day for PFOS) in lines 5878 to 5880 (figures 14 and 15) according to the estimated exposure in chapter 3.4.3. Please use consistent assumptions for the two modelling approaches. This would also clarify whether or not persons exposed to levels twice the TWI at age 1 to 10 would end up with blood levels higher than 12.6 ng/mL at age 35.	
German Federal Institute for Risk Assessment (BfR)	150	3.4.4. Derivation of a Health Based Guidance Value	Lines 5822-5918 (p 154) The TWI is calculated based on a proportion of 28% PFOA+PFNA and 72% PFOS+PFHxS for both background exposure and serum levels at 35 years of age. What would happen if the proportion of PFOA were higher, but we still respect the TWI of 8 ng/kgBW/wk? To explore this, we look at the case that the TWI is exhausted exclusively from PFOA, such that mothers have a plasma concentration of 12.6 ng/ml and the children from 1 year of age consume 8 ng/kgBW/wk of PFOA afterwards. The resulting plasma concentration can be seen in Fig 1 of the present document (see uploaded file) to exceed the NOAEC of 31.9 ng/ml at the critical time between 0 and 1 years of age. We would like to see this explained in the uncertainties. It is possible for populations exposed from e.g. drinking water with high PFOA (but still under the TWI for food and	It is correct that the mixture approach taken may not cover all possible scenarios and, in some circumstances (exceptions), the resulting HBGV may not prevent that serum levels exceed the $BMDL_{10}$ (see response to comment number 148). This is also due to the fact that there is insufficient information on the potency of PFNA and PFHxS. Based on several comments from stakeholders this uncertainty was partly addressed using BMD modelling to derive the HBGV. The use of a BMDL takes the underlying uncertainty of the data into consideration and the resulting HBGV is as a consequence more protective (and should partly cover the points raised here). It should also be stressed that the CONTAM Panel considers the decreased vaccination response a risk factor for disease rather than a disease.

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			drinking water together) to achieve serum concentrations such that their breast-fed children exceed the NOAEC of 31.9 ng/ml. Lines 5938-44 (p 158) NTP performed a meta-analysis on immunotoxicity associated with exposure to PFOA and PFOS (NTP 2016). We suggest integrating this analysis into EFSA's new risk assessment; this would be supportive for EFSA's conclusion, especially as the key study (Abraham et al., 2020) was not considered by NTP. The NTP concludes that PFOS/PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOS/PFOA NTP performed a meta-analysis on immunotoxicity associated with exposure to PFOA and PFOS (NTP 2016). We suggest integrating this analysis into EFSA's new risk assessment; this would be supportive for EFSA's conclusion, especially as the key study (Abraham et al., 2020) was not considered by NTP. The NTP concludes that PFOS/PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOS/PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans.	Regarding NTP (2016), see response to comment number 143.
			Line 5951-6012 (p 158ff) The TWI protects breastfed infants as presumably the most vulnerable and at the same time highest exposed age group. Please clarify the interpretation of exceedances of the TWI in different age groups regarding health concerns. TWI exceedances in men, elderly and very elderly do not result in health risks to breastfed infants. Health risks are also unlikely in population groups with intakes that do not result in serum levels above 31.9 ng/ml. Please, describe in more detail for which population group an exceedance of the TWI results in increased health risks concerning e.g. - Relevance for elderly population (immune system senescence) (WHO/IPCS 2012) - Relevance for boys and (young) men	The TWI is set to protect all population groups, including breastfed children. It appears that infants are the most sensitive group because they show the highest serum levels in the general population. However, effects on the immune system may also occur at older age as shown by a small study in humans and in mice. The following is stated in 3.4.1: *'Although performed with a small group and thus not suitable for deriving a critical serum level, the study by Kielsen et al. (2016) with 12 adults shows that the decrease in vaccination response may also occur at much older age and low serum levels of PFASs (median levels for PFOA, PFHxS and PFOS of, respectively, 1.7, 0.7, 0.4 and 9.5 ng/mL). This is supported by the results from mice studies that were performed with adult animals (see Section 3.3.3.5).'*

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			- Relevance for women who will not have a(nother) child	It is unclear if a serum level of 17.5 ng/mL would be the Reference Point in adult age groups and more studies are needed. However, exposure below the TWI ensures that serum levels in adults are 2- to 3-fold lower than this value.
			If for some population groups (e.g. see above) the TWI is not relevant due to the underlying effect, what is the critical effect and the TWI for these population groups?	The setting of different HBGVs for specific age groups is interesting but is beyond the scope of this risk assessment.
			Line 5940 (p 158) Typo: please insert "it" between "if" and "is"	Regarding line 5940: Thank you for noting, the text has been adjusted.
German Federal Institute for Risk Assessment (BfR)	151	3.5. Risk characterisation	Line 5951-6012 (p 158ff) The TWI protects breastfed infants as presumably the most vulnerable and at the same time highest exposed age group. Please clarify the interpretation of exceedances of the TWI in different age groups regarding health concerns. TWI exceedances in men, elderly and very elderly do not result in health risks to breastfed infants. Health risks are also unlikely in population groups with intakes that do not result in serum levels above 31.9 ng/ml. Please, describe in more detail for which population group an exceedance of the TWI results in increased health risks concerning e.g. - Relevance for elderly population (immune system senescence) (WHO/IPCS 2012) - Relevance for boys and (young) men - Relevance for women who will not have a(nother) child	The available studies do not allow the derivation of a critical serum level for older children and adults. There is one small study showing effects in adults with low serum levels and also the studies with mice are performed with older animals. The levels in infants are likely to be the highest in the normal population. In older age groups applying the same TWI scenario, the serum levels are estimated to be 2.5-fold lower than during infancy and are likely to be protective. Further studies are required to confirm that the TWI is low enough to protect older age groups. The CONTAM Panel does not agree that the TWI is not applicable or conservative for older age groups.
			If for some population groups (e.g. see above) the TWI is not relevant due to the underlying effect, what is the critical effect and the TWI for these population groups?	Based on the available studies, adults seem also sensitive to these effects.
			Line 5978 (p 159) "This exposure of breastfed infants corresponding to the TWI could be a better starting point." The meaning of the sentence is not clear. Which starting point is addressed here? Please rephrase.	In the updated opinion, the intake of infants at TWI exposure of the mothers is now shown in a graph based on the PBPK models.

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			Line 5985 (p 159) Please clarify why it is difficult to evaluate the impact of the exceedance of the TWI by toddlers and other children. If the exposure does not result in blood levels higher than 31.9 ng/ml, it should not be of health concern. Using toxicokinetic modelling should answer this question quantitatively.	The TWI in principle applies to all age groups. It is explained why for infants the exposure should not be compared with the TWI. For young children it is shown that the NOAEC observed in the Faroes children is not exceeded, even when assuming a two-fold higher exposure than the TWI.
			Line 5987-5988 (p 159) Please explain the assumption that some of the children have an exposure that could result in adverse effects on the immune response. This assumption should be supported by toxicokinetic modelling results.	Toddlers and children show a P95 exposure between 19 and 96 ng/kg bw per week, the latter being up to 4- to 22-fold higher than the TWI. This would result in serum levels of 30 to 144 ng/kg bw at 5 years of age, being 1- to 5-fold higher than the critical serum level in the study from the Faroe Islands. Hence the conclusion that most of the children may be at risk.
			Line 5998 (p 159) After clarifying for which age groups a TWI exceedance results in a health concern, only these age groups should be included in table 22 (which is set up to compare the exposure with the TWI). Alternatively, exposures of age groups (like infants) that should not be assessed based on the TWI should be marked with an explanatory footnote.	See above. The CONTAM Panel does not agree that the TWI only applies to part of the population.
			Line 6000 ff (p 159) In this paragraph, the serum levels (of mothers at 35 years) corresponding to the TWI are compared to biomonitoring data. Obviously, these biomonitoring data are used as supporting data. However, the serum levels mentioned are the primary outcome of the derivation of a HBGV, and the TWI results from additional modelling steps (with its own uncertainties). Furthermore, the exposure assessment (for comparison with the TWI) has relatively large uncertainties, primarily due to missing occurrence data allowing a valid assessment. Taking this together, the uncertainties of a risk assessment based on a comparison of TWI and exposure assessment are relatively high. Therefore, it makes sense to give the biomonitoring data a much higher relevance regarding the question what	The CONTAM Panel agrees that biomonitoring data of persistent contaminants may allow a better risk assessment than the assessed exposure assessment. However, the CONTAM Panel routinely compares exposure with the established HBGV but thinks that the comparison of $BMDL_{10}$ serum levels with the exposure offers the best opportunity to evaluate the uncertainty in the risk assessment, both the derivation of the TWI and the exposure assessment. Furthermore, the CONTAM Panel considers the PBPK model as rather accurate and did not apply additional uncertainty factors to derive the TWI. At this stage it is difficult to conclude on recent trends both in serum levels and exposure, since recent data are missing to perform such a comparison.

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			proportion of the population is at risk. For these comparisons, actual data are needed, and considering the decrease in (PFOA/PFOS) serum levels, those used from 2007/2008 onwards may be not the best ones for the risk assessment. It should be a recommendation (5.) to perform more PFAS biomonitoring studies, especially in women of childbearing age.	
German Federal Institute for Risk Assessment (BfR)	152	3.6.1 Uncertainty in exposure estimates	General for this chapter: BfR agrees that all decisions made within the exposure chapters are reasonable and well justified given the challenge of handling an exposure assessment with such a large fraction of left-censored data. In Figures 6 through 9, the draft opinion provides valuable information about the relative proportion of exposure contribution for the individual food groups across the different member states. As the main contributing food groups vary between member states as well as between the specific compound, we would like to request that this information is provided in numerical form, probably best in the form of an additional annex.	The numerical information has been added, please see response given for comment number 131.
			General for this chapter: Please add as an uncertainty that only the diet was included in the exposure assessment and that non-dietary sources were not considered.	The CONTAM Panel agrees that it is important to inform that only diet was included in the exposure assessment, and this is already mentioned in Section 3.6.1 '*In the current exposure assessment, neither non-dietary exposure nor exposure to precursors have been considered, but both of these are included when biomonitoring data are used as a measure of exposure. This contributes to an underestimation of the exposure.*'
German Federal Institute for Risk Assessment (BfR)	153	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	Lines 6100 ff (162) It is not a satisfactory situation that the TWI was derived from the HiB antibody response given that the dose response relation with the sum of PFAS is the least well-established (compared to tetanus antibodies, see data in the Appendix, and see discussed BMDU/BMDL ratio in line 6100 ff). We request a more thorough explanation. It was shown in Abraham et al. (2020) that there is a statistical correlation between PFOA levels and Hib, tetanus and diphtheria	The CONTAM Panel is aware that in the paper by Abraham et al. (2020), only PFOA levels show a significant association, but the additional data show that this also applies for the sum of 4 PFASs. Furthermore, the study by Grandjean et al. (2012) shows a significant association for PFOS and in animal studies the lowest Reference Point was obtained with PFOS. Therefore, EFSA decided to derive a TWI for the sum of 4 PFASs, now based on

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			antibody titers, but not for PFOS. This correlation should be shown and discussed in the opinion. Starting from that, it could be rationalised that due to evidence from other studies (Grandjean et al., 2012), PFOS (and PFHxS/PFNA) were additionally included in the TWI derivation.	the $BMDL_{10}$ obtained for diphtheria in the study by Abraham et al. (2020).
			Lines 6127 ff (p 162 ff) A more detailed and quantitative sensitivity analysis should be made for the PBPK modelling, since this is crucial for TWI derivation and hence the risk assessment. The quantitative change of the TWI should be estimated for the following parameters (and combinations thereof) to get a better understanding of the amount of uncertainty in the TWI resulting from assumptions and modelling:	Additional information has been added to the PBPK Appendix showing the sensitivity of the models for variation in a number of parameters, including body weight, daily milk volume, transfer rate and related % decline per day and variation in PFAS composition.
			Lines 6174 ff (p 163 f) Transfer of PFAS from serum to milk: Impact of the transfer rate and the decline in milk concentration on the TWI should be calculated.	This has been added to the PBPK Appendix.
			Lines 6198 ff (p 164) Factors related to half-life of PFAS in serum: Impact of modelling parameters, which contribute to the half-life of PFAS in humans (e.g. absorption, distribution in several tissues, excretion and reabsorption), on the TWI should be calculated with regard to the uncertainty of the PBPK-modelling. For quantitative estimation of uncertainty, for example, the 95% confidence interval of the TWI resulting from the 95% confidence interval of the model could be calculated.	These parameters were fitted by the developers of the model (Loccisano et al., 2011) and the CONTAM Panel concluded that these parameters should not be varied.
			In course of that it should be kept in mind that the biomonitoring studies cited in the EFSA, 2018 opinion (for PFOS, PFOA) and cited in the current opinion (for other PFAS, Table 16 and 17), which were used for validating the model, were often conducted on persons significantly older than 35 years. For those persons, the actual PFAS uptake over their lifetime is quite uncertain.	The CONTAM Panel is aware of uncertainties in the published models but is not in the position to further optimize the models. Furthermore, application of one-compartment models based on half-life and Vd show quite similar results (see EFSA CONTAM Panel, 2018). However, EFSA made a recommendation for further improvement of PBPK models.
			Lines 6198 ff (p 164)	

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			Impact of age at which mothers give birth: Please give a quantitative estimation of the change of the TWI under the assumption that mothers give birth at the age of 20 (or 40). This seems to be important for assessing the risk arising from TWI exceedance in different population groups.	The CONTAM Panel agrees that this can be relevant but such a detailed approach on exposure and resulting serum levels at different ages seems beyond the scope of a risk assessment. For deriving the TWI EFSA used an age of 35 years for a mother, at which age the serum levels are close to steady state.
			Lines 6198 ff (p 164) Impact of breastfeeding on mothers: Please give a quantitative estimation of the change of the TWI under the assumption that the mother was not breastfed herself (or only for a shorter time) as an infant.	Based on the half-lives of the 4 PFASs and the increase in body weight, it is evident that being breastfed as infant does not affect the serum levels at 35 years of age. This is even true for children and is now added to the opinion (updated Figures 14 and 15).
			Lines 6174 ff (p 163) In this paragraph, the question was raised whether the PBPK model is able to correctly describe the values measured in the key study (Abraham et al. 2020). It was concluded that the assumed maternal levels at birth (measured values of mothers not having breastfed) are too high, and possible explanations were discussed. However, this is misleading as it was not realized that the breastfed children of the study (Abraham et al. 2020) received mother's milk for 7.4 months only (mean), and not for 12 months. To test the model, the levels of mothers not having breastfed the child (PFOA 4.9 ng/mL, PFOS 17.2 ng/mL) were taken for the maternal levels before pregnancy. After exclusive breastfeeding for 7.4 months (study average) and oral intake of 0.66 and 1.66 ng/kg bw per day after weaning for PFOA and PFOS, respectively, the levels at the age of 353 days (mean age breastfed children) were predicted to be 13.9 ng/mL (PFOA, measured: 16.8 ng/mL) and 18.3 ng/mL (PFOS, measured: 15.2 ng/mL). Therefore, the prediction of the sum (32.2 ng/mL) was in very good agreement with the measured sum (32 ng/mL), but the model parameters of PFOA and PFOS may require further fine-tuning and validation for the accumulation during breastfeeding.	The ability of the applied PBPK to predict serum levels is now further addressed in Appendix M.

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German Federal Institute for Risk Assessment (BfR)	154	4.3 Critical effects, dose response assessment, derivation of HBGV (conclusions)	6475-6477 (p 172) As discussed at EFSA's online stakeholder meeting from March 12, 2020 – a 2-year carcinogenicity study in Hsd:Sprague Dawley® SD® rats was performed by NTP (Dec. 2019, draft) which could not be considered by EFSA (draft published after deadline of EFSA opinion). However, NTP concluded that under the conditions of this study, there was clear evidence of carcinogenic activity of PFOA in male rats and some evidence of carcinogenic activity of PFOA in female rats. Assuming a non-genotoxic MoA, could EFSA please check and communicate (e.g. in a FAQ document), whether the derived TWI for the sum of PFOA, PFOS, PFNA, PFHxS would be protective for carcinogenicity in humans?	Please see response to comment number 48
German Federal Institute for Risk Assessment (BfR)	155	4.4 Risk Characterisation (conclusions)	Line 6490-6493 (p 173) Please clearly state if the LB exposures of toddlers and other children represent a health concern for this age group. The resulting serum levels in toddlers and other children are not assumed to be higher than 31.9 ng/ml, keeping in mind that an exposure of breastfed infants at 220 ng/kg bw per week (25 times the TWI!) does not result in critical serum levels in infants. Line 6499 (p 173) Please add "These exceedances of the TWI at LB exposure estimates indicate a health concern for infants after a long breastfeeding period.	Please note that the CONTAM Panel stated that the TWI is not applicable to infants. From 3.4.4: *'This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant that are associated with effects on the immune system. In doing this, the higher exposure of breastfed infants is already taken into account in the derivation of the TWI, and this TWI is not applicable for infants.'* The statement was slightly changed, i.e. the exposure of infants should not be compared with the TWI, since the higher exposure is already taken into account when deriving the TWI. Regarding for which age groups the TWI is applicable; please see answers to comment numbers 127 and 150.
German Federal Institute for Risk Assessment (BfR)	156	5. Recommendations	Line 6529 (p 174) Biomonitoring: Toxicokinetic information indicates (also stated on p. 69) that short chain PFCAs are preferentially excreted in urine. Therefore, the recommendations should include a statement that urinary biomonitoring methods should be used (or developed if not yet available) to monitor these substances.	The CONTAM Panel agrees that more studies on paired human samples are important to identify the relevant matrices for biomonitoring of various PFASs, and has thus added this in the recommendations.

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German Federal Institute for Risk Assessment (BfR)	157	Appendix K – Additional information on the study from Abraham et al. (2020)	Table K.1 - line 10721 (p 436) Please complement the column captions of Table K.1 or the corresponding table legend with the units of the mean values. There are currently two columns labelled "(group) mean" without specifying the measurements. Moreover, it should be denoted that antibody titers are given as log values. Otherwise the decrease of Hib antibody titers by more than 50% mentioned e.g. on page 150 is not comprehensible. Since it is indicated for PFAS sums that values are given as means this should also be done for the antibody titers.	The Table K.1 in the opinion has been updated as suggested.
German Federal Institute for Risk Assessment (BfR)	158	-	*The attachment submitted by this commenter during the public consultation (in relation to comment 148 and 149) is listed and available in Annex F of this Technical report.*	-
Ministerium für Ländlichen Raum und Verbraucherschutz Baden Württemberg	159	Summary	p. 4 line 160 Unfortunately, EFSA has so far not commented on the short-chain PFC compounds.	As described under comment number 39, the CONTAM Panel restricted the assessment to the 4 PFASs included in the human studies. More data are needed on short-chain PFASs with regard to the critical effects.
Ministerium für Ländlichen Raum und Verbraucherschutz Baden Württemberg	160	3.3.1.4 Transfer from feed to food	p. 73 line 2357 to 2377 This section refers to existing feeding studies, whereby bioaccumulation factors are derived for certain PFC compounds. As regards the practical implementation of feed monitoring, it would be useful if these data could be used to derive animal species-specific transfer factors (direct correlation between the concentration in the respective animal tissue and the concentration in the feed) in order to estimate provisional action thresholds for feed with the knowledge available so far (possibly under worst case	Please see response to comment number 71.

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			assumptions - such as continuous feeding with contaminated feed until fattening).	
Ministerium für Ländlichen Raum und Verbraucherschutz Baden Württemberg	161	3.6.1 Uncertainty in exposure estimates	p. 160 line 6043 to 6046 Section 3.6.1 explicitly points out that short-chain PFAS are increasingly used worldwide and that exposure estimates based on previous data do not reflect the current situation. Toxicological assessments of short-chain PFAS, which occur mainly in plant foods from contaminated areas, are urgently needed for the evaluation of foods on the market, but also for the assessment which foods should be excluded from the market (see also the following point).	Please see above response (comment number 159).
Ministerium für Ländlichen Raum und Verbraucherschutz Baden Württemberg	162	Annex B – Distribution of analytical results	Table B.4 It seems as EFSA has evaluated only PFAS analytical data up to and including 2016 for its draft opinion, and from Germany even only data up to and including 2015, but there are many other current analytical data from the years after that which should definitely be considered (see also the previous point). Analysis data from the official food control authorities are currently collected by the BVL via the BVL data reporting portal. However, only PFAS analysis data from the official food control authorities are compiled. In Baden-Württemberg, there is another large data pool of relevant PFAS investigation results with partly clear positive findings of PFAS compounds, which should be taken into account. This is the pre-harvest monitoring data of the Agricultural Technology Centre (LTZ) Augustenberg. This special monitoring program was initiated after a large-scale PFAS contamination case that has led to significant PFAS findings in agricultural products (for details see https://rp.baden-wuerttemberg.de/rpk/Abt5/Ref541/PFC/Seiten/default.aspx	All data received up to the inclusion date of 16th May 2018 have been included in the Opinion. EFSA agrees that more recent occurrence data would have been of interest and added in the recommendations that the exposure assessment should be updated when analytical data obtained with more sensitive methods become available, also to reduce the uncertainties in the exposure assessment. Risk assessments of EFSA cover the general population and not incidents or hot spots, unless specifically requested by the Commission.

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			subchapter "Landwirtschaft" (Agriculture), in German language. Agricultural crops from this region have been tested for their PFAS content for several years before harvest and are not marketed as food if they contain higher levels of PFAS. For this reason, these findings have not been included in the database of the official food control authorities. Samples from pre-harvest monitoring with conspicuous PFAS findings are, however, in principle products suitable for food use (e.g. fruit, vegetables, cereals). The only reason why they have not been placed on the market as food is that the authorities have actively prevented the placing on the market on the basis of the pre-harvest analyses in dialogue with the farmers. In our view, the data from the Baden-Württemberg pre-harvest monitoring are therefore important data that document the uptake of mainly short-chain PFAS compounds in plants from contaminated land and should therefore be made available to EFSA for its risk assessment. Thus, the pre-harvest monitoring data is transferred to EFSA in addition to the data from the official food control authorities.	As stated in the public consultation explanatory note, due to time constraints, EFSA cannot use additional occurrence data submitted during the public consultation for the dietary exposure assessment in this risk assessment.
American Chemistry Council	163		Please see attached comment*. **The attached comment submitted by this commenter during the public consultation is listed and available in Annex G of this Technical report.*	For questions related to immune effects in humans, please see replies to comment numbers 102, 183 and 178. For questions related to immune effects in experimental animals, please see replies to comment numbers 169 and 183, and regarding involvement of PPARa the reply to comment numbers 147. For questions related to mixture approach, please see replies to comment numbers 116, 177 and 178.

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National Institute for Public Health and the Environment (RIVM)	164	Abstract	General comments We appreciate the vast amount of work done by EFSA for this draft opinion and would like to provide our comments below.	Noted
National Institute for Public Health and the Environment (RIVM)	165	Summary	Throughout the opinion it is not clear whether equipotency is assumed on external or internal dose level. We suggest to clarify this in the relevant sections. NB due to TK differences, equipotency can only apply on the external or internal level, not on both.	In principle, equipotency could apply to both internal and external exposure. The factor that is important is toxicokinetics. The NOAECs serum levels are converted to weekly exposure using a PBPK model. Hence the equipotency for the 4 PFASs used for the TWI applies to external exposure.
National Institute for Public Health and the Environment (RIVM)	166	1.3.5. Previous risk assessments	Lines 1138-1146. The reference to the RIVM report on relative potencies for PFASs seems to be missing. We request to add it and hereby provide the details. Zeilmaker M, Fragki S, Verbruggen E, Bokkers B, Lijzen J, Mixture exposure to PFAS: A Relative Potency Factor approach. RIVM Report 2018-0070, DOI 10.21945/RIVM-2018-0070, https://www.rivm.nl/bibliotheek/rapporten/2018-0070.pdf	Thank you, the reference has been added.
National Institute for Public Health and the Environment (RIVM)	167	2.2.1. Dietary Exposure assessment	L1334-1336: 'To assess the combined exposure to PFOA, PFNA, PFHxS and PFOS the mean occurrence value of each of the four PFASs for each food category was summed and considered to be the combined occurrence of these PFASs within the food category'. No explanation was given for the approach to sum up the individual PFAS's mean occurrence values to the total of the 4 PFASs, not in the text or at Tables 4 and 5 (paragraph 3.1.2, page 43 and 44). We therefore suggest including an explanation for using this approach instead of a sample based approach (i.e. not all samples were always analysed	The methodological sections explain how the exposure assessment was performed. Since this approach does not clearly affect the exposure assessment performed by EFSA to these persistent compounds, it was decided not to add additional explanations in the section on exposure assessment. It is evident that it would be better to have analytical data obtained with more sensitive methods to decrease the large uncertainty in the assessment. This we have clearly stated in the recommendations. See also response to comment 162.

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			for all 4 PFASs). In addition, EFSA is requested to elaborate on the impact of this approach on the exposure, e.g. uncertainties.	
National Institute for Public Health and the Environment (RIVM)	168	3.1.2. Analytical results	Lines 1499-1503: "The mean occurrence values calculated on the final dataset for the selected 17 PFASs in each food category and used in the exposure assessment are displayed in Annex A, Table A4. Table 4 shows the mean levels in a number of food categories for the 4 PFASs selected based on biomonitoring data, being PFOA, PFNA, PFHxS and PFOS. Table 5 shows the mean concentrations of these PFASs in a number of fish species (see Appendix A and Annex A, Table A4 for more fish species)." Table A4 contains occurrence data for PFOA, PFNA, PFHxS, PFOS, and the sum of 4 PFASs for 80, 80, 76, 74 and 80 food groups, respectively. Tables 4 and 5 in the report present in total 25 food groups. Thus, Tables 4 and 5 present different information than Table A4 in Annex A.	It was decided to only provide occurrence data on a selected group of food products, allowing some comparison of important food groups. Since fish is in general the most important source, it was decided to include also a table on the most important fish species. It is clearly stated that more detailed information can be found in the Annex A.
			It is unclear on what basis the food groups presented in Table 4 and 5 were selected, and if/how these were used for the exposure assessment. RIVM would like to suggest presenting the data used for the exposure assessment in the report and, if doing so, to present both occurrence data from the individual PFAS and the sum of the 4 PFASs (which is currently not the case in Table 4 and 5). Furthermore, we suggest adding a remark to the Tables that the total PFAS used for exposure assessment have been obtained by the presented individual mean occurrence values (see our comment to 2.2.1).	This section is on occurrence. The analytical values used for the exposure assessment are those presented in the Annex A Table A4 as stated in 3.1.2. Details on the type of PFASs can also be found in the Annex A. A remark was added that the mean levels of individual PFASs was used in the exposure assessment, as also explained in the methodology section
			In addition, in the above phrased text EFSA mentions that the 4 PFASs were selected based on biomonitoring data. This reads somewhat confusing as it could be interpreted	This was rephrased.

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			that biomonitoring data were presented in Table 4, we suggest rewording '4 PFASs selected based on biomonitoring data, being PFOA, PFNA, PFHxS and PFOS' to 'food categories for the 4 PFASs selected for this risk assessment, being PFOA, PFNA, PFHxS and PFOS', and possibly add a reference to lines 5699-5702.	
National Institute for Public Health and the Environment (RIVM)	169	3.3.3.5 Immunotoxicity	These comments also relate to paragraph 3.4.3.1 and Appendix H. We acknowledge that the animal studies on immune effects of PFAS indicate a large uncertainty in the data set, both between and within studies. We subjected the data from the studies of Peden-Adams (2008) and Dong (2009) to BMD analysis. See uploaded file for the analysis. We have the following questions concerning the animal data. • EFSA states that the animal studies (Peden-Adams 2008; Dong 2009) are subject to high uncertainty in their dose-response due to an unavailable zero-dose group. However we note that in these animal studies the response at the lowest 2-3 dose groups levels off enabling an estimate of the background response (parameter a). The accuracy of the estimate of the BMD-CI could be further improved by setting constraints to the background response, which could be obtained from historical data (i.e. experiments with different substances than PFASs, but with similar experimental setups). See figures in the uploaded file. Would EFSA consider to obtain information on background and perform DR modelling using this information to constrain the background? In line 5834 EFSA concludes that the BMD CIs are too wide based on the Peden-Adams and Dong study. Indeed the	The CONTAM Panel decided to base its assessment on human studies with support from studies in mice. However, at an earlier stage the CONTAM Panel asked the advice of the Scientific Committee WG on BMD modelling, specifically on these two studies and the study by Grandjean et al. (2012) on children. The WG concluded that no suitable BMDLs could be derived from these studies and advised the Panel to apply a NOAEL approach. Applying constraints in the modelling is not according to the Guidance and cannot be applied in the available PROAST models. Furthermore, using background immune responses measured by the PFC test from other studies is not adequate since the absolute number of PFCs very much depends on the protocols used (i.e. timing of sensitization and reagents such as the SRBC batch and complement used, strain of animals) for which reason the absolute values in responses (including those at background exposure levels) are highly variable between studies.

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			BMD-CI for the Peden-Adams study is very wide. For the Dong study our analysis showed that the BMD10-CI spans a factor of about 70. Could EFSA indicate when a CI should be considered too wide? Does EFSA consider the BMD10-CI obtained from the Dong study too wide? • Could EFSA indicate what should be considered a toxicologically relevant critical effect size (CES) to use in a BMD analysis of the immune effects in animal studies?	At present there is no guidance on the acceptable size of the confidence interval. But remember that the WG BMD advised not to use the outcome of the modelling. Furthermore, the Peden-Adams study would be the preferred study based on the lower NOAECs the lower serum levels in the controls and better methodology (experiment performed twice and with more optimal immunization period). Based on the advice of immunological experts there was no reason to deviate from a CES of 5%. Whereas a person that is sufficiently vaccinated may not suffer from a minor decrement in his or her vaccination response, this is different on a population basis, as not everyone is optimally protected after vaccination. In addition, and even more importantly, a decreased vaccination also indicates a general decreased immune response. Also, on an individual level the immune system may have a certain reserve capacity, but on a population basis this reserve capacity is limited. Infectious diseases are evident in the population, and in those suffering from infections the immune system has clearly not been able to mount a sufficient response. As part of the population suffers from infectious diseases, the reserve capacity at the population level has been used and further reduced functionality of the immune system will lead to further increment in infectious diseases. IPCS and NTP consider any significant decrement in the immune system, as evidenced by a reduced vaccination response, as relevant. Applying the BMD approach, EFSA would choose to use the 5% CES for animal studies. It should be noted, however, that a critical appraisal of this value in the context of the database is always necessary (*EFSA Scientific Committee, 2017. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. doi:10.2903/j.efsa. 2017.4658).*

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National Institute for Public Health and the Environment (RIVM)	170	3.3.4.4.2 Vaccination response	These comments also relate to paragraph 3.4.1. While the study by Abraham et al. (2020) has been properly designed and conducted, we identified some issues that possibly could influence the results of the statistical analyses and their interpretation.	
			First, the study population consists of separate subpopulations that differ or could differ with respect to plasma PFAS and antibody concentrations or potentially confounding variables such as socioeconomic factors: 1) a group of children from Berlin vs. a group from Ilsenburg, a dioxin hotspot in a more rural area; 2) a group of breastfed children vs. a group of predominantly formula-fed children. There is also overlap between these two categorizations because the average duration of exclusive breastfeeding was shorter in the group from Ilsenburg. Since breastfeeding may influence childrens' antibody concentrations through antibodies in breastmilk, breastfeeding should be considered as a potentially confounding factor. On the other hand, care should be taken to avoid overadjustment because the type of feeding is also a determinant of plasma PFAS concentrations. To increase insight in the influence of the subgroups on the results of the regression analyses, stratified analyses and additional adjustments for group (breastfed/formula-fed and Berlin/dioxin hotspot) could be performed.	The CONTAM Panel acknowledges that some populations from different areas in Germany contribute to the generalisability of the study. Furthermore, the data are supported by those from Norway, Denmark, the Faroe Islands and animal studies. The formula fed children were added to increase the power of the study and show no significant differences in the antibody titres with the breastfed children in the lower quantiles.
			Second, the results of the regression analyses are presented as scatter plots with a regression line. To evaluate the uncertainty of the results, we would like to see the CIs of the regression line and the regression coefficients of the final model and the models with stepwise adjustment for potentially confounding variables (including subgroups as indicated above).	The CONTAM Panel thanks RIVM for the additional analyses provided on the uncertainty of the 'knee'. The results support the use of the BMDLs obtained with the individual models in PROAST, which are now the basis for deriving the Reference Point.
			Third, the use of regressed values of (log-transformed) antibody concentrations at the time of vaccination (to account for differences in the time since last vaccinations	This adjustment seems important and was justified by the analysis of the data by Abraham et al. (2020).

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			and the number of vaccinations) comes with the assumption that antibody concentrations are highest at the time of vaccination and then decrease over time in a (log-)linear fashion. We request to add a discussion on the validity of this assumption. Fourth, animal studies indicate that there may be a difference in susceptibility between males and females. To improve the interpretation of the results it is suggested that EFSA explores whether the use of sex as a covariate influences the statistical analysis of the data. In conclusion, we suggest that these issues be further investigated and that more information is presented to be able to better judge the associations between plasma PFAS and antibody concentrations.	This analysis was not performed by the authors. However, contrary to some animal species, the current database does not suggest gender differences in the toxicokinetics of PFASs in humans (except the effect of pregnancy and breastfeeding), which at least partly underlies observed sex differences in effects.
National Institute for Public Health and the Environment (RIVM)	171	3.4.1 Critical effects	The 4 PFASs (PFOA, PFNA, PFOS and PFHxS) were chosen as a basis for the HBGV because 'At present, these are the PFASs that contribute most to the levels observed in human serum (see section 3.3.2 on biomonitoring). Although some other PFASs like PFBA and PFHxA also contribute significantly to the exposure (see section on current exposure), these compounds show much shorter half-lives in humans and thus much higher exposure is required to obtain similar serum levels as the 4 PFASs selected.' (lines 5701-5702). However, this implies that (other) PFASs with long half-lives but low levels of exposure, such as PFDA, and possibly PFUnDA (lines 2255-2259), could still be relevant for humans. EFSA concluded that PFDA induced effects on the immune system (line 6335). This conclusion was not drawn for PFUnDA, which has only been shown to aggravate allergic reactions in vivo (line 5549), an effect that may not need to be immune-related. PFDA is detected in food products (e.g Table 3), but according to the current (limited) occurrence data it does not contribute significantly to the calculated total exposure. However, the data show that the public is exposed to this PFAS.	The CONTAM Panel agrees that other PFASs than the 4 included in the TWI may also affect the immune system, and that especially the ones that accumulate in humans are of concern. However, it was decided to include only the ones that were detected in the serum of children in the two studies, also because these were the ones detected more generally in human blood. The CONTAM Panel did not apply relative potency factors based on effects in liver because there is too much uncertainty on a common MoA behind both immune effects and those on the liver. More studies are required to derive RPFs for immune effects. An additional sentence was added that other PFASs may also affect the immune system and that especially the ones with long half-lives are of concern. However, the CONTAM Panel considers applying an additional UF too conservative, also based on the type of endpoint.

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			In Figure 10, it is shown that PFDA (and PFUnDA) contribute relatively 2-3% to the medians of PFASs in blood. In the Grandjean studies, PFDA was detected in children's serum (although at low concentrations) and inversely associated with antibody response (line 4307, 4336, 4458). In the Abraham study used for the POD, PFDA was found to be exclusively or predominantly below the LOQ. The samples for the Abraham study were taken in 1997-1999, and 'Generally, after the year 2000, the concentrations in serum/plasma… …of PFNA, PFDA and PFUnDA have increased' (lines 6284-6286). Therefore, even though PFDA was not detected in children's serum in 1997-1999, it is a possibility that PFDA currently does contribute to the internal exposure to PFASs in the young children, and to a reduced vaccination response in this age group. This can be especially relevant if PFDA is more potent than the 4 selected PFASs. Based on our 2018 assessment of RPFs (Zeilmaker et al., 2018) and our equipotency analysis of various endpoints in the 28-day NTP study (NTP, 2019a, b, see our comments to 3.4.2), PFDA classifies as a potent PFAS, with a similar high potency as PFNA (up to 20-fold the potency of PFOA). We understand that, because PFDA was not detected in children's serum, it cannot be linked directly to the decreased vaccination response in the Abraham study and as such be included in the POD. Therefore, to reckon with the possibility that PFDA (and possibly other undetected PFASs) could contribute to the reduced vaccination response, we suggest applying an additional uncertainty factor to the POD in deriving the HBGV. This uncertainty factor would then account for the possibility for the exposure to other PFASs, especially PFDA which has long half-life, shows immunotoxicity and high potency on other endpoints. (We also refer to our proposals for using an uncertainty factor in our comments to 3.4.2 and 3.4.3).	

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National Institute for Public Health and the Environment (RIVM)	172	3.4.2 Mixture Approach	EFSA did not derive relative potency factors for the 4 selected PFASs because 'there are no comparative studies that provide reliable insight in the relative potencies' (line 5770) and 'Due to the lack of a clear MoA, results of in vitro studies can help to improve our understanding but cannot be used to derive potency factors at this stage' (line 5805). EFSA therefore assumed equipotency as (lines 5808-5811) 'The absence of a clear MoA is not a reason for not applying a mixture approach. As a pragmatic approach, the CONTAM Panel decided… …to assume equal potencies by default for these four PFASs on immune outcomes.' On several occasions EFSA does recognize that equipotency is implausible. For example in lines 5095-5107 where it is stated that 'In general, [….] most endpoints are somehow affected by all PFASs, and differences seem merely [?] due to kinetics and relative potencies.', and in L5706 of the draft opinion where it is noted that: 'the available data are insufficient to derive potency factors for the different PFASs, although such differences in potency are likely to exist.' Although the modes of action of PFASs behind various effects (on liver, immunology, development, and endocrine disruption (thyroid)) are still unknown, it is clear that the potencies of the various PFASs differ. This was (amongst others) shown by RIVM (Zeilmaker et al., 2018) for the potencies of several PFASs, including PFHxS, PFOS, PFOA and PFNA, to elicit effects on liver endpoints. Preliminary results of an analysis performed by us for the current public consultation on the data of 28 day NTP studies (NTP 2019a, b) show that similar potency differences are also present in other organ weights, hormone levels, clinical chemistry, white blood cell parameters (but not in RBC parameters) and pathology endpoints (results of this analysis can be shared and discussed with EFSA upon request). Generally, the PFASs were ordered similarly in their ability to cause effects on these endpoints. Regarding the 4 PFASs discussed in the draft opinion, this order (from low to high potency) is PFHxS, PFOA, PFOS, PFNA. Also the order of magnitude of the	The CONTAM Panel fully agrees that RPFs should be derived for different PFASs but in the absence of comparative studies or clear indications that liver and immune effects share a common mechanism, it seems difficult to agree on RPFs. As a pragmatic approach, the CONTAM Panel decided to assume equal potencies for the 4 PFASs. See also response to comment number 171.

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			potencies (compared to PFOA) is similar to the potencies based on liver effects, only. It should be noted that especially PFNA cannot be considered equipotent to the other three PFASs, but is generally a factor 6 to 20 more potent, based on the available data. Could EFSA therefore consider not to assume equipotency (of the 4 PFASs) but to apply the RPFs obtained from liver effects (which seem generally applicable)? Besides the fact that this assumption would be a better reflection of the (albeit limited) evidence in scientific literature, an additional benefit of this approach would be that the sum group of PFASs can be extended from 4 to all the PFASs with a derived RPF (see L1138), including PFDA, which also may be considerably more potent than PFOA. Alternatively, we suggest applying an additional uncertainty factor in the derivation of the TWI to account for differences in potency of the 4 included PFASs. (We also refer to our proposals for using an uncertainty factor in our comments to 3.4.1 and 3.4.3). L5771-5789: This paragraph indicates that the serum levels of the 4 PFASs are correlated. EFSA concludes that it is uncertain whether PFOA has a higher potency. However, it can also be concluded that it is uncertain whether all 4 PFASs contribute (equally) to the effect. It may also be the case that only 1 or a combination of 2 or 3 PFASs contribute to the effect. It is not possible to conclude from the epidemiological studies that all 4 PFASs contribute equally. We suggest to include this notion in this section. L5790: 'Another issue is whether to base relative potencies on applied dose or e.g. serum levels (internal dose).' Relative potency factors (RPFs) should match the metric of the exposure data, i.e. when the exposure is expressed externally (e.g. ng/kg bw/day) then external RPFs are needed. The same is true for internal doses (e.g.	

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			biomonitoring data). Hence, we suggest to delete the last sentence (L5794-5795).	
National Institute for Public Health and the Environment (RIVM)	173	3.4.3.1 Effects on the immune system	In the draft opinion several studies in humans and animals are presented that indicate effects of PFASs on the immune system. Analysis of both the human and animal studies indicate a large uncertainty in the available data sets. EFSA chooses to base its POD on data from the Abraham study. In Lines 5830-5834 EFSA concludes: "Modelling of the data from both the Faroe Islands and Germany with the BMD modelling programmes normally applied by EFSA (PROAST and BMDS) resulted in wide BMDL-BMDU intervals, as a consequence of extrapolating to zero exposure (well below the lowest observed serum levels). Therefore NOAECs were derived in the present opinion, based on the distribution of the participants into quintiles". Our questions concerning the Abraham study are addressed under section 3.3.4.4.2 . ANOVA We noted several issues with the derivation of the NOAEC (appendix K and L): • In the pairwise comparison the quantiles Q2 and up are compared to Q1. As EFSA also indicates in line 6111, this means that the outcome of the comparison depends on the choice of the number of quantiles. We would like to emphasize that this is an important uncertainty, as illustrated by the different results from the quintiles versus deciles evaluations. • It is not clear how the NOAECs are defined. For anti-Hib only Q4 has a p-value <0.05, while Q5 is not significant (p>0.05). Why did EFSA choose Q3 as NOAEC? • The NOAECs do not relate to a particular effect size. Could EFSA provide the effect sizes (incl CI) at the NOAECs to give insight in the severity of the effect at NOAEC? • The NOAECs ignore the uncertainty in the data. Could EFSA describe the uncertainty in the NOAECs and provide a confidence interval?	The CONTAM Panel is aware of the uncertainties around the use of NOAELS and recommends the use of BMD modelling. However, the Scientific Committee WG on BMD modelling considered the two animal studies and the study on children from the Faroe Islands as not suitable for deriving a BMDL that could be used for deriving a TWI. This seemed also the case for the study by Abraham et al. and therefore the CONTAM Panel initially used a NOAEC based on quintiles. However, following the comments by RIVM, an analysis with PROAST by the authors at the request of the CONTAM Panel showed that BMDLs can be derived for antibody titres against tetanus and diphtheria. Therefore, it was decided to use the lowest BMDL for deriving the TWI. It was observed that model averaging provided much lower BMDLs, but also that part of the curves did not fit the data accurately. This is most likely caused by the lack of serum levels close to zero and the inherent issues with extrapolation beyond the lowest serum levels. Still, these curves determine the lower value for the confidence interval (BMDL). Therefore, it was decided to use the lowest BMDL of the individual models. It is also important to stress that the significance values obtained with the data for PFOA support even more an effect on the vaccination response (Abraham et al., 2020). However, other studies, including those in animals, support that PFOS also affects the immune system, and based on some additional studies it seems likely that also other PFASs can do this. Weighing all the evidence, the CONTAM Panel decided to include the 4 PFASs, for which data were available in the human studies, in the dose-response modelling. The differences in the mean antibody titres in the different quintiles compared to the first one are now added in the Appendix. For the BMD modelling it was decided to apply a CES of 10% to take into account the high variability in the titres.

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			BMD analysis (see uploaded file) We note that according to the EFSA guidance, in case model averaging is not possible, selection of the BMDL from a limited number of models (as was done in the past) is still acceptable. We have subjected the data from the Abraham study (scanned from Figure K1-K3; accuracy > 99%) to dose-response modelling and found that for the anti-Hib response no dose-related trend could be identified in the four model types (exp, hill, invexp and logn) i.e. these data are not considered suitable to derive a POD for this endpoint, not by BMD analysis. For anti-Tet and anti-Diph, a marginally significant trend (model 3 describes the data better than the NULL model) was found which was mainly driven by the data point with the highest measured concentration. After removing this data point from the data set, no significant trend was found. This analysis indicates that the dose-response information is not robust. In addition, for some of the models a trend could only be established at the expense of setting extreme (constrained) values for model parameter "d" (see the EFSA guidance on DR modeling in 2017). Model averaging, including all data points, also produces usable BMD confidence intervals , but the bootstrapping process generates a large number of non-informative DR curves, which are rejected. Hence, the outcome that this model averaging shows is inconclusive. All in all, the BMD analyses indicate that the Abraham data do not produce a strong and reliable POD. Analysis of knee-function Just like other types of regression models (constant response model, linear response model, quadratic response model) a bi-linear response model gives a valid statistical description	However, there is no consensus on which decline should be regarded as adverse within a population.

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			of the data (results available upon request). In the case of a Diph vaccination response, a bi-linear response model was a statistically significant improvement compared to the other models. However, since the dose-response curve was mainly determined by the small number of data for high doses, more data are necessary to conclude that the bi-linear model is a relevant type of regression model for PFASs or other compounds. Conclusion/summary Overall, we acknowledge, based on our evaluation of the data using different analytical approaches, that the database contains marginal dose-response information showing quantitative PFAS effects on the human immune system. Unfortunately, this dose-response information has been shown not to be robust. The database is weak and the use of a different dataset to derive a POD for setting a HBGV would be preferable. However, we note that also the animal studies on immune effects of PFASs do not provide a robust POD without additional information. Therefore, we suggest EFSA to include in the opinion a thorough discussion on the uncertainty included in the PoD, e.g. the confidence interval around the NOAEC. We also suggest applying an uncertainty factor to address and compensate for the uncertainty in the POD. (We also refer to our proposals for using an uncertainty factor in our comments to 3.4.1 and 3.4.2).	This conclusion suggests that effects on the immune system in both animals and humans cannot be used as the basis for the risk assessment. The CONTAM Panel thinks that the overall body of evidence clearly points to the fact that PFASs affect the response to an antigen and considers this effect as a risk factor. In addition, these effects have been observed at similar serum levels. The interindividual variation in response seems large but the overall picture is clear. Based on the comments of the stakeholders, the CONTAM Panel reconsidered the approach and derived a BMDL based on the study of Abraham et al. (2020). The animal data were used as supportive evidence. The CONTAM Panel strongly supports ongoing work to improve the BMD modelling software to allow better modelling of these types of datasets.
National Institute for Public Health and the Environment (RIVM)	174	3.4.4. Derivation of a Health Based Guidance Value	Lines 5945- 5948: 'The CONTAM Panel decided that no additional uncertainty factors need to be applied, because the NOAEC is based on infants which are expected to be a sensitive population group, as is true for many immunotoxic chemicals. The CONTAM Panel also took into account that the NOAEC is based on risk factors for disease rather than disease'. Referring to our comments to the sections 3.4.1, 3.4.2 and 3.4.3, we suggest that EFSA considers applying an	Children may indeed be the most sensitive population but at present there are no data that support this with respect to PFASs. However, children are likely to show higher serum levels at infancy. The CONTAM Panel considered this option but for several reasons decided not to apply additional uncertainty factors, the

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			uncertainty factor to address and compensate for the uncertainty in 1) the POD, 2) the assumption on equipotency and 3) the limitation of the assessment to only 4 PFASs, while it is known that other PFASs contribute to the total exposure. Lines 5955-5957 ' The CONTAM Panel noticed that this TWI is protective for the other potential critical endpoints (increase in serum cholesterol, reduced birth weight and high serum levels of ALT) considered in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018).' To illustrate the protection for other endpoints, we suggest including in the opinion an overview of effect doses/PODs for those other endpoints.	major one being that the endpoint is not an adverse effect by itself. However, the TWI was further lowered based on the BMDL modelling, providing further protection for the whole population and children in particular. For other potential critical endpoints the CONTAM Panel would like to refer to the previous Opinion, keeping in mind that causality of several of these effects has been questioned and requires further studies. Furthermore, also for these potential effects, a mixture approach should be applied, which would increase the obtained BMD values. However, the derivation of a confidence interval around the BMD was criticized and further work is needed on BMD models for this type of data sets. Furthermore, data on the sum of PFASs were in general not available for these endpoints.
National Institute for Public Health and the Environment (RIVM)	175	3.6.1 Uncertainty in exposure estimates	EFSA addresses a number uncertainties in the exposure estimates. We agree that these are very important. Table 23 "Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of PFASs in food" presents an overall view. However, EFSA only uses ' +/-,' '+' or '–' to indicate whether the sources over or under estimate the exposure, but does not give a weight (magnitude) of the uncertainty sources' influence on the exposure. A '+' does not necessarily compensate a '–' . We request EFSA to give an overall conclusion on whether the uncertainties result in an over- or under estimation of the exposure.	The EFSA Scientific Committe adopted a recent Opinion on uncertainty analysis (EFSA Scientific Committee, 2018) including a more quantitative approach. However, application of this approach should be considered from the start of a new Opinion since it may require specific adaptations in the various calculations. This Opinion was started before it was decided to change the approach. As stated under Table 23 of the opinion '*The CONTAM Panel has used LB exposures for the risk characterisation as it considers that the UB exposure assessment results in a large overestimation, also based on comparison of the critical serum level in mothers with the data from biomonitoring (see Section 3.5 risk characterisation).'*
National Institute for Public Health and the Environment (RIVM)	176	-	*The attachments submitted by this commenter during the public consultation (in relation to comment 169 and 173) are is listed and available in Annex H of this Technical report.*	-

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The 3M Company	177	Abstract	Taking the critical concepts for hazard characterization and risk assessment developed over the years in the evaluation of research studies, it is imperative to apply a weight of evidence approach for dose response, consistencies across studies and species, and known biological plausibility. • The EFSA CONTAM Panel (2020) report is 459 pages. The critical effect study by Abraham et al. (2020) became publicly available approximately 3 weeks ago although the CONTAM Panel had access to the actual study data for many months prior in order to conduct their sum PFAS (PFOA, PFOS, PFHxS, and PFNA) analyses. These EFSA sum PFAS analyses were not published in the critical study of Abraham et al. EFSA provided limited space (5000 characters including spaces) to respond to any individual section of their report. Although we have separated out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage EFSA to read our full comments, which we have uploaded as two separate files. The first file contains our written comments, tables, and figures and appendices A and B. The second file contains appendix C. • Summing and applying equal potency for PFOA, PFOS, PFHxS, and PFNA is not supported by science. • To date, the available data on the PFOS and PFOA immunotoxicity studies have not demonstrated concordance and, the immune-related data for PFHxS and PFNA are even more limited. • Given that EFSA has acknowledged that "a clear mode of action of immunotoxicity by PFOS and PFOA has not been established" (c.f. section 3.3.5.4), it is unclear how these inconsistent findings from the animal and human studies can be used by EFSA to develop a guidance value. Thus, the TDI proposed by the EFSA CONTAM Panel (2020) should only be considered, at best, provisional.	The CONTAM Panel agrees with these principles and thinks that this applies on the most critical effect, being those on the immune system. These effects were repeatedly shown in animal models and also in human studies. The CONTAM Panel agrees that the uncertainty in the human studies seems larger but using human data prevents the use of additional uncertainty factors that overall would have resulted in a much higher uncertainty and lower TWI. The CONTAM Panel obtained a preview of the study by Abraham et al. (2020), but made sure that all the relevant data were available in the draft Opinion. Data on individual infants were not available for the CONTAM Panel, and the authors were asked to provide additional calculations on the sum of 4 PFASs both for the draft Opinion and in response to the comments of the Stakeholders. Based on structural similarities and similar persistence in humans, the CONTAM Panel considers it very likely that more PFASs will affect the immune system, although with different potencies. At this stage the data do not allow the derivation of relative potency factors for the relevant PFASs. The CONTAM Panel feels that not applying a mixture approach would seriously underestimate the risk of PFASs and applied a default potency factor for the four PFASs taken into account. It was recommended to perform comparative studies to improve the risk assessment. The CONTAM Panel recommended more studies to improve this approach, which may lead to revision of the HBGV in the future.

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The 3M Company	178	Summary	The EFSA CONTAM Panel (2020) proposed a TDI based on findings from a cross-sectional epidemiological study of 101 one-year old children (Abraham et al. 2020) that reported a statistically significant association between serum PFOA and reduced antibody responses to Haemophilus infuenzae type b, tetanus, and diphtheria but found no significant associations with PFOS, PFHxS, or PFNA. While the cross-sectional study can demonstrate statistical correlations, it cannot be used as a proof of causation.	Based on the studies in animals and those by Grandjean et al. (2012) and Granum et al. (2013) with children, the CONTAM Panel considers that there is enough evidence to conclude that PFOS causes immuntoxic effects. Focussing only on PFOA in the study by Abraham et al. (2020) suggests that the effect is only due to PFOA. As explained in the Opinion a pragmatic approach was taken including evidence for an effect for PFOS in other studies as well as toxicokinetic and structural similarities between the four PFASs.
			EFSA CONTAM Panel obtained access to the authors' data in order to conduct an analysis involving the sum of these four perfluoroalkyls. A No Observable Adverse Effect Concentration (NOAEC) of 31.9 ng/mL at the age of 1 year was derived for the sum of PFOA, PFNA, PFHxS and PFOS (sum PFAS), based on the inverse association between the sum PFAS and antibody titers against Haemophilus influenzae type b (Hib).	Noted
			There are uncertainties in the analyses of the food data reported and the estimation of this newly proposed TDI – some of which the CONTAM Panel (2020) acknowledged. Food data were primarily submitted by only 3 countries with 92% of the results reported below LOD/LOQ. Biomonitoring and food concentrations of PFAS continue to decline. The conclusion of an adverse effect is based on a significantly reduced antibody response related to only PFOA exposure in the Abraham et al. study with no evidence that there was an increased risk of infection in these one-year old children. The CONTAM Panel conducted the PBPK sum PFAS (data obtained from Abraham et al.) models on grouped, not continuous data.	Noted, see below.
			There are several technical issues with EFSA's statistical approach which reflect faulty decision-making or implementation. These issues have resulted in the NOAEC of 31.9 ng/mL. The following points highlight these concerns:	

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			1) The reported exposure data (i.e., serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups. 2) The determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach would be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis.	The CONTAM Panel agrees with the issues around using a NOAEL and strongly supports the use of BMD modelling. However, the CONTAM Panel considers the approach as a valid alternative when BMD modelling does not provide a BMDL that can be used as the Reference Point. Initially this seemed the case for the human studies. Two stakeholders provided information that this was not correct and the data were analysed with PROAST, showing that a BMDL can be derived for antibody titres against tetanus and diphtheria based on the individual models, but not with model averaging. The lowest BMDL from the individual models was now used to revise the TWI.
			3) Albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse association between serum levels of the sum of these 4 PFASs and antibody titers against Haemophilus Influenza type b (Hib)" (cf. page 7 of the draft scientific opinion).	The CONTAM Panel considers that both animal and human studies show that PFASs are immunotoxicants. The CONTAM Panel asked the data provider to check the statistics and it was confirmed that the p-values for the ANOVA were indeed not correct (now corrected in the Appendix of the Opinion). However, the statistics on the differences between quantiles were correct. As mentioned before, the CONTAM Panel now used BMD modelling for deriving the Reference Point. BMD modelling using PROAST also confirms that there is a significant dose response based on fitting either the Hill or exponential models.
			4) The statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach and constrained the overall data interpretation. Furthermore, summing and applying equal potency for PFOA, PFOS, PFHxS, and PFNA is not supported by science.	The CONTAM Panel does not agree and thinks that based on structural similarity and persistence, at least these four PFASs should be grouped and assumed to affect the immune system. The issue on the relative potencies is relevant and future studies should aim to derive such factors. The absence of these factors is not a valid argument for not applying a mixture approach. Similar is true for a lack of insight in the MoA.

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			To date, the available data on the PFOS and PFOA immunotoxicity studies have not demonstrated concordance and, the immune-related data for PFHxS and PFNA are even more limited. The critical laboratory animal study cited by EFSA (Peden-Adams et al., 2008) does not provide sufficient evidence for PFOS-related immune suppression; and the other epidemiological data cited do not support reduced resistance (via titer measurement) with tetanus for either PFOA or PFOS and they provide imprecise reduced antibody estimates for diphtheria. Collectively, the epidemiologic data do not suggest an increase propensity for infection. Given that EFSA has acknowledged that "a clear mode of action of immunotoxicity by PFOS and PFOA has not been established" (c.f. section 3.3.5.4), it is unclear how these inconsistent findings from the animal and human studies can be used by EFSA to develop a guidance value. Thus, the TDI proposed by the EFSA CONTAM Panel (2020) should only be considered, at best, provisional. We trust these scientific emphases will be taken into consideration by EFSA with the final assessment.	See previous reply. Peden-Adams applied a routine test for studying effects on immune response. Human studies show a general trend for a decreased vaccination response. No epidemiological studies specifically designed to investigate associations between PFAS exposure and infections are available. Such studies are difficult to perform, as the prevalence of e.g. tetanus infection is low, and consequently the numbers of subjects to be included in such studies need to be large. Nevertheless, there are some indications in humans of increased infections, as described in the opinion, and supported by at least one publication in animals, referred to in the opinion as well as by the stakeholder himself. The CONTAM Panel therefore does not agree that the information is inconsistent.
The 3M Company	179	3.3.2.2 Time trends	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 76, lines 2504-2511: Based on the summary data provided on lines 2504 – 2511 of the draft scientific opinion, EFSA should provide the actual concentration trends and percentage of declines in graphical formats instead of the text data presented in Appendices B1 – B4 of the draft scientific opinion. Clearly, PFOS and PFOA have declined in the European general population since 2000. This can best be seen graphically.	The CONTAM Panel agrees that it would have been helpful to illustrate time trends graphically, but as many studies and several PFASs are considered and would have had to be included it was considered not feasible.

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The 3M Company	180	3.3.3.2.2 PFSAs (Effects following repeated exposure)	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file.	
			Although EFSA (2020) lists Chang et al. 2017 in the references, EFSA does not discuss this insightful toxicological study. Nor was it mentioned or referenced in the earlier EFSA (2018) report. EFSA should acknowledge that Chang et al. undertook a six-month oral dose study with PFOS administered to male and female cynomolgus monkeys, with scheduled clinical assessments through 1 year, in order to evaluate markers for coagulation, lipids, hepatic, renal, electrolytes, and thyroid-related hormones. There was a time-matched control group as well as 4 weeks of baseline values for the dosed groups. The low dose group (n = 6/sex) received 1 single K+PFOS dose (9 mg/kg) with the highest mean serum concentration measured at 68000 ng/mL. The high-dose group (n = 4-6/sex) received 3 separate doses (11 – 17.2 mg/kg) during the six-month treatment phase with the highest mean serum concentration measured at 165000 ng/mL. Liver needle biopsies performed two months after completion of the study showed the highest mean liver PFOS concentrations at 112000 ng/g. At end of study all the animals were considered healthy, gained weight, and released back to the colony. Throughout the entire study, there were no K+PFOS treatment related changes in serum liver enzymes, serum BUN or creatinine. There was a decrease in serum total thyroxine without a concomitant change in in the clinically-relevant TSH and free T4. Authors considered the decreased total thyroxine observed was likely due to competitive displacement by PFOS with of thyroxine and its subsequent increased metabolism and elimination. The most notable observation in this study was upon treatments, the mean serum total cholesterol was decreased by approximately 4 – 12% at 1 and 3 weeks post-dose when compared with mean time-matched control or baseline values. The reduction in	The study of Chang et al. (2017) was published after the literature deadline of the 2018 CONTAM Panel Opinion, but has been carefully considered in the current Opinion (Appendix E, Table E.2). The focus of this Opinion is to identify adverse effects of PFASs for hazard identification and risk assessment. However, in the study by Chang et al. (2017) cynomolgus monkeys showed no PFOS-related effects except decreases in serum cholesterol. To mention all parameters, which remained unchanged in the various studies included in the Opinion, is outside the scope of the opinion.

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			cholesterol was used to determine a lower-bound fifth percentile benchmark concentration (BMCL1SD) of 74000 and 76000 ng/mL in male and female monkeys, respectively.	
			Page 89, lines 2914-2920: 3M respectfully disagrees with EFSA's interpretation on the results of liver findings from Chang et al. (2018). As the primary contributing authors of this study, our conclusion on liver effects remained the same in that "the microscopic findings in the liver were considered to be consistent with an adaptive response" (cf. Chang et al. 2018). This conclusion was based the weight of evidence and corroborative data. The observation of hepatocellular lipid vesicles was of uncertain genesis and significance, it was likely to be associated with the lipid metabolic changes induced by nuclear receptor-mediated effects. The necrotic hepatocytes were some minimal randomly distributed foci of necrosis in the liver (including one male mouse from the control group), but this type of necrosis is occasionally seen as an incidental background finding in laboratory rodents. Even though there were increased incidence of single cell necrosis noted in the highest dose group males, however, RT-PCR transcript markers associated with cellular stress were not increased in these mice.	In Table 8 on the histopathological alterations in the liver, Chang et al. (2018) reported dose-dependent and significant ($p<0.05$) increases in the incidence of cytoplasmic vacuolation in females and of microvesicular fatty changes and single-cell necrosis in males. Furthermore, Table 9 shows a significant ($p<0.01$) dose-dependent 2-fold increase in the serum level of alkaline phosphatase in males, being indicative for some cholestatic damage in the liver. The CONTAM Panel interpreted these findings as evidence for hepatotoxic effects of PFHxS.
			Page 90, lines 2964-2968: 3M respectfully disagrees with EFSA's conclusion that "alterations in the kidney" was documented repeatedly from toxicity studies with PFBS, PFHxS, and PFOS. Kidney has not been identified as a target organ for repeated dose studies with PFHxS or PFOS. Even under the most enduring dosing administration of 2-year chronic bioassay in rats with PFOS, there was no PFOS treatment-related effects in kidneys (Butenhoff et al. 2012 Toxicology 293 1-15). With PFBS, the kidney alteration effects were only noted under extremely high dosing conditions where the microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage. This may have been due to a response to high concentrations of PFBS	The NTP study (2019) on PFBS reported a dose-dependent significant increase ($p<0.01$) in relative kidney weights of female rats starting already at the lowest dose applied (62.5mg/kg bw/day). This was followed by papillary necrosis in the highest dose group (1,000 mg/kg bw/day) in 4/10 females ($p<0.05$). To conclude, there is evidence for adverse effects on the kidney already at moderate doses of PFBS. The summary was clarified and modified to: `*In summary, an elevated absolute and relative liver weight was the most sensitive endpoint for PFBS, PFHxS and PFOS. No repeated dose toxicity studies were available for PFHpS and PFDS. Disturbed lipid metabolism, necrosis and inflammation in the liver were mostly seen at higher dose levels. Also disturbed*

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			passing through the kidney and into the urine; because PFBS is excreted rapidly in the urine with a serum elimination half-life of several hours in rats, almost 90% of each daily administered dose would be present in the daily urine output of these rats.	*thyroid hormones and alterations in the kidney (PFBS only) were documented. For details see Appendix E.'*
The 3M Company	181	3.3.3.3.2 PFOA (Developmental and reproductive toxicity)	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 91, lines 3029-3030: For PFOA, 3M respectfully disagrees with EFSA's conclusion that "the most sensitive developmental effect observed is delayed and impaired development of the mammary gland upon prenatal and early postnatal exposure". In addition to the high-level summary stated above in the main text, the technical comments that 3M submitted to ECHA in 2014 regarding about the study by Macon et al. (2011) are attached (as Appendix C in the full comment to this submission) to illustrate the fact that there are numerous areas of scientific uncertainties on this particular topic. Macon et al. (2011) was considered (and then withdrawn) by ECHA for its ANNEX XV restriction proposal on PFOA in 2014	Delayed or impaired development of mammary glands after prenatal and early postnatal PFOA exposure has been observed consistently across different studies and mouse strains in several experiments, as described in Table F.1. The LOAEL is based on findings in three different studies confirming each other, of which Macon et al. (2011) was one of them. The CONTAM Panel acknowledges uncertainties in developmental studies on endpoints less frequently addressed, such as mammary gland development, but the consistency in the findings across studies is reassuring.
The 3M Company	182	3.3.3.3.4 PFSAs (Developmental and reproductive toxicity)	Page 99, lines 3425-3443: In the mouse developmental study (identified as Feng et al. 2017 in the draft scientific opinion) total T4 and total T3 alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected, especially when TSH, the primary diagnostic indicator for thyroid hormone status, was normal. Feng et al. did not provide sufficient information for a full interpretation of thyroid status. There are also several technical concerns with this study: o The observations from Feng et al. (2017) need to be validated. There were eight individual serum hormones measured and reported by Feng et al. (2017) based on the blood samples collected from the newborn mice; each hormone was	The CONTAM Panel reported the findings in the study and based the NOAEL on delay in development, and not on decrease in thyroid hormones, although the authors suggested that as the reason for the developmental delay**.** The CONTAM Panel takes note that some additional information in the description of the methods in the paper by Feng et al. (2017) would have been useful for more precise review of the

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			measured using commercial ELISA kits from USCN Life Science Inc. According to the manufacturer's information (see https://www.cloud-clone.us), each ELISA kit requires 50 uL of serum sample volume. A newborn mouse pup is quite small in size (approximately 1 gram), so it is not clear how Feng et al. was able to measure all the hormones with such a limited blood volume. To better understand, 3M consulted with Charles River Laboratories who concluded that, if they were to repeat the Feng et al. study, they would need at least 75 dams per dose group to achieve the blood sample volume required for the specified hormone measurements. Feng et al. only had 30 dams per dose group.	data (e.g. format of the ELISA kits, sampling of blood and tissue orientation for histological examination).
			o The discrepancies between mouse and rat developmental data need to be addressed. The developmental endpoints from the short-term gestation exposure study in mice by Feng et al. (2017) were vastly different than the outcomes from the full 2-generation study in rats by Lieder et al. (2009). These differences need to be properly assessed before a scientific conclusion can be made. Key observations included effects reported by Feng et al. lacked dose-responses; the effects from 200 mg/kg-d were usually similar in magnitude to 500 mg/kg-d. The study design and PFBS dosing regimen by Lieder et al. was more rigorous than Feng et al. in terms of treatment duration, doses, and direct treatments to developing fetuses and pups during sensitive life stages, see the comparison table in Appendix C in the full comment to this submission.	The CONTAM Panel reported both the rat and mice developmental data available for PFBS. The reasons for discrepancies between the studies are not known, and could be both species- and study-specific. For instance, comparative toxicokinetic parameters between rats and mice were not identified by the CONTAM Panel for PFBS (Appendix C) and possible species differences were thus not elaborated. The selection of the experimental model (e.g. not including male offspring, way of dosing) is responsibility of the author. The CONTAM Panel is aware of the different methodological approaches taken in the papers by Feng et al. (2017) and Lieder et al. (2009).
			In addition, it is not clear why Feng et al. did not include male offspring in their evaluation. The female mouse offspring in the Feng et al. study were not directly dosed with K+PFBS, but the reported adverse developmental outcomes occurred in these female mouse pups (e.g., reduced body weight and changes in reproductive organ morphology). In contrast, female rat offspring (from Lieder et al. 2009) were exposed to PFBS during gestation and lactation and were directly dosed with PFBS (at higher dose levels than the Feng et al. study) after weaning and into	In the material and method section of the paper by Feng et al. Authors refer to adults. The CONTAM Panel assumes that authors were interested in adult outcomes of developmental exposure and therefore not investigated pubertal offspring at PND30.

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			their adulthood. There were no developmental effects noted in the female rat pups in Lieder et al.	
			o Regarding alterations in ovary and uterus-related data reported by Feng et al., there were several technical details not provided by the study authors which precluded a meaningful interpretation of the data.	Technical issues are commented above.
			☐ Evaluation was reported for female pups at PND 60 only, not on PND 30 and not for dams (who were directly dosed with PFBS). ☐ "Impaired" development reported by Feng et al. was based on decreased surface area (on microscopic slides) and limited morphological measurements. Surface area can be also attributed from different sectioning location (of the tissue). Feng et al. did not address how this was controlled among different animals. In addition, Feng et al. only provided relative organ-to-body weight data and no organ-to-brain weight data or absolute organ weight data. ☐ Feng et al. did not take body weight into consideration when interpreting estrous cycle data even though they are related (Bermejo-Alvarez et al. 2012). ☐ In Feng et al. (2017), despite changes in female reproductive organ morphology, functional aspects of reproduction appeared not to be affected according to study authors (i.e., maternal body weight, maternal body weight-gain, and various pregnancy outcomes).	The paper by Bermejo-Alvarez et al. (2012) is on reproductive consequences of diet induced obesity and of rather limited relevance here. There was no influence of treatment on body weight of dams observed by Feng et al. (2017).
			Page 100, lines 3464-3477: 3M respectfully disagrees with EFSA's conclusion that "the most sensitive reproductive endpoint for PFHxS exposure was reduced litter size". Even though there was a slight but statistically significant decrease in the mean live litter size in the 1 and 3 mg/kg-d groups, the toxicological significance of this finding is unclear based on the lack of a clear dose-response; pup to implant ratios were similar among control and the treated groups; and the lack of other negative effects on development or reproduction. Similarly, at similar	The CONTAM Panel agrees that the results on reproductive endpoints with PFHxS exposure are hard to interpret; still, reduced litter size was a relevant outcome to report. The lack of dose response and that pup to implant ratios were similar is also reported. However, please note that the reproductive endpoints are not seen as the most sensitive for PFHxS, as is also stated: *'In general, gestational exposure to PFHxS produces effects in offspring animals at doses which were equal to or higher than*

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			serum PFHxS concentrations, live litter size was not affected in Wistar rats (Ramhoj et al. 2018 Toxicol Sci 163 579-591).	*those inducing responses in parental animals (see also Section 3.3.3.2).'*
The 3M Company	183	3.3.3.5.1 PFOS (Immunotoxicity)	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 107, lines 3729-3767 and page 110, lines 3876-3878: Study by Peden-Adams et al. 2008 does not provide sufficient evidence for PFOS-related immune suppression Among a myriad of outcomes that have been reported for PFOS in the laboratory animals, EFSA considered the study by Peden-Adams et al. (2008) to be the critical toxicological study for supporting its current scientific position. The study by Peden-Adams et al. reported immunosuppression based on decreased antibody IgM TDAR responses in splenocytes in mice (upon exposure to SRBC antigen) and accordingly to EFSA, this may lead to reduced resistance to infection. The decreased IgM antibody response in splenocytes upon SRBC challenge alone is insufficient to support the notion that PFOS caused an immunosuppression effect. Antibody responses should be done corroboratively in other key immune organs such as thymus, lymph node, bone marrow, and serum. Peden-Adams et al. (2008) did not evaluate antibody response in thymus from the same animals. In a separate cohort of animals, they did report decreased serum IgM level, however, these animals were challenged once with trinitrophenyl conjugated to LP (TNP-LPS) instead of SRBC hence it was difficult to compare the results among these data; given two different antigens were used and the antigenic effect induced by TNP-LPS could be mediated by either B cells or T cells. In addition, in the NTP (2016) monograph which declared that "PFOS is presumed to be an immune hazard to humans", it concluded that PFOS	The CONTAM Panel is happy with the conclusion of the 3M company, as stated in the 4th paragraph, that PFOS suppresses antibody responses in mice, as supported by a number of studies. The CONTAM Panel does not, however, agree with the notion that one might deduce from this comment that there is doubt on the interpretation of these data in terms of the relevance. The plaque forming cell assay (PFC) is seen by the international immunotoxicology community as a prime parameter to show immunosuppression. It measures the number of plasma cells from the spleens of rodents immunized to SRBCs, producing IgM specific to SRBC. IgM lyses the SRB cells after binding complement and the plaques of lysed cells, representing individual plasma cells producing the IgM are counted. IgG can be measured in a indirect PFC assay, but this approach is more cumbersome and the outcomes are more variable. An alternative is to measure specific antibodies by ELISA. Whereas many studies have been performed using the PFC assay, more experience is being gained using ELISA, and also using alternative antigens than SRBC. An advantage of SRBC over soluble antigens such as TNP-OVA is that these do not require degradation by phagocytes, hence a component of the immune system is not addressed in that assay. Whereas it is true that for vaccination responses in humans usually IgG responses are measured, it is not true that IgM is

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			exposure is associated with suppression of NK cell activity in animals. However, Peden-Adams et al. (2008) actually reported increased NK cell activities. Even though the conclusion that PFOS suppresses antibody responses in mice is supported by a number of other studies which also showed that exposure to PFOS at various life stages can affect the IgM levels (Dong et al., 2011; Keil et al., 2008; Peden-Adams et al., 2008), it is important to recognize that the antibody titers (to antigen vaccinations) are primarily of the IgG antibody isotype, not IgM. Peden-Adams et al. (2008) did not evaluate IgG response in mice; and even when IgG was evaluated in mice by others such as the studies by Dong et al. (2011) and Qazi et al. (2010), the secondary IgG response was not appropriately induced to elicit a bona fide memory response as only a single antigen challenge was used in these studies. A later study by Lee et al. (2018) evaluated serum IgG1 and IgE levels in mice that had been challenged by multiple ovalbumin treatments before PFOS administration and again one more time after PFOS administration, they did not see a suppression of serum IgG1 or IgE. Rather, increased serum IgG1 and IgE were reported. Page 107, lines 3747-3752: It is worth noting that Peden-Adams et al. (2008) also reported non-dose-response changes in splenic CD4/CD8 subpopulations, however, this specific finding has not been replicated in the subsequent publications coauthored by Dr. Peden-Adams (Fair et al., 2011; Mollenhauer et al., 2011). As discussed by Fair et al. (2011): Moreover, data from this study confirm that numbers of CD4+ cells were within normal ranges. This contrasts a previous report from this laboratory where absolute numbers of CD4+ cells were decreased in female B6C3F1 mice at 0.1 mg/kg [total administered dose] but not at 1.0 mg/kg TAD using a similar 28-day exposure regimen (Peden-Adams et al., 2008). This previous observation was from a single experiment, whereas in this study, the experiment was	not produced. There will be an isotype switch from IgM to IgG. For resistance, depending on the pathogen, both may be relevant. The measurement of IgG is done as a check of sufficient immune responses and protection to the specific pathogen, even if the real correlate for protection is not always clear and established by convention. Isotypes such as IgG1 and more so IgE are associated with allergic responses. Ovalbumin is a well-known allergen, used in many studies to investigate allergic responses, and is indeed a good inducer of IgG1 and IgE. The immune system is a system of checks and balances, governed by regulator T cells, steering towards either Th1 or Ths type of responses, among others, and an increase in IgE titers cannot simply be used as an argument to refute decreased protective antibody responses. The CONTAM Panel is surprised by the comment on measurement of antibody responses in the thymus. The thymus is not an organ where antibody responses take place, at least not in any relevant amount. It is also not easy, although not impossible, to measure antibody responses in lymph nodes, blood, especially not using PFC. However, as the spleen is a prime organ included in the circulation, the response there is considered representative for the capacity of the organism to mount a response. The CONTAM Panel likes to stipulate that the vaccination responses measured in the studies in humans to which the opinion refers are IgG titres, hence the CONTAM Panel concludes therefore that the animal data by Dong and by Peden-Adams support the decrement in functionality of the system in humans. The CONTAM Panel agrees with the 3M company that the information on numbers of different cell types and as well as one study in which NK activity was measured is not strong, and should have been repeated. In general terms it should be mentioned that numbers of immune cells may differ, and that changes in numbers of cell types may serve as an alert for an

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			repeated twice for absolute numbers and three times forpercent changes with all experiments yielding the same results. The effect previously reported was not dose-responsive and is likely to be a transient effect. Overall, these data indicate that T-helper cells, B-cells, and MHC-II+ cells were not selectively eliminated.	effect on the immune system, but do not necessarily indicate functionality, in contrast to antibody production. The CONTAM Panel has therefore not based its conclusions on immune cell numbers.
			Page 108, lines 3775-3776: The animal evidence for an effect of PFOS on disease resistance/infectious disease outcome is weak. EFSA relies essentially on one study by Guruge et al. (2009) which reported decreased survival in PFOS-treated mice infected with a mouse-adapted strain of influenza virus (H1N1). However, the study did not evaluate viral titers to conclude whether anti-viral activity was affected by PFOS treatment.	The CONTAM Panel acknowledges that the evidence on resistance to infections is not as strong as it is for antibody responses, as stated in the opinion. In addition to some evidence in humans, the 3M Company is right in mentioning the Guruge study. The CONTAM Panel agrees that measuring viral load in the animals would have strengthened the study. Meanwhile, the CONTAM Panel also notes that mortality due to virus infection is a rather crude measure, and also requires a considerable decrement in resistance in order to lead to mortality. Mortality as an outcome is therefore not very sensitive, and it is not unreasonable to presume that effects on viral load might have produced effects at lower doses of PFOS.
The 3M Company	184	3.3.4.1.1 Birth weight	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file.	
			Page 113-115, lines 4091-4170 (unless otherwise noted): 3M respectfully finds that EFSA misinterpreted the Meng et al. (2018) study and its relationship to the Steenland et al (2018) meta-analysis of birthweight and PFOA. EFSA writes of the Meng et al. 2018 study "For example, the large (n = 3,535) study by Meng et al. (2018) observed consistent inverse associations between PFOS and PFOA and birth weight. In that study, samples were drawn in early pregnancy (first trimester), which should minimize the risk of confounding by physiological changes in pregnancy (Steenland e tal. 2018)." In prior lines (4044 – 4053), EFSA, however, correctly acknowledged about the Meng et al. study (as clearly discussed in Meng et al.), "First trimester	Regarding misinterpretation of the Steenland meta-analysis, the CONTAM Panel disagrees. The overlap between the 1,400 subjects included in his analysis and that the new data are based on 2,120 women is clearly stated. These new data (n=2,120) and inclusion of these data if an updated meta-analysis were to be performed, would lead to slightly different estimate and perhaps conclusion. What the new data adds is to confirm that the inverse association observed in the previous study (whose chemical analyses have been criticised, see comment number 19 above) are confirmed. There is also stronger indication of possible adversity in terms of preterm delivery. The strength of this study which is a mixture of previously analysed and newly analysed

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			serum samples from 3,535 women were analyzed for PFOS and PFOA, and from 2120 women for other PFASs. This included 1400 women who had previously been described in another publication (Fei et al. 2007) (emphasis added)." What EFSA failed to also acknowledge is that the Fei et al. (2007) study of 1400 women was, in fact, included in the Steenland et al. meta-analysis of birth weight and PFOA. And Fei et al. was part of the stratified first trimester analysis as seen in Figure 3 of the Steenland et al. paper. Thus, to argue, as EFSA writes on lines 5672-5675 that, 'the association with reduced birth weight might at least partly be explained by changes in the physiology during pregnancy, although a recent study seemed to strengthen the causality of the effect (Meng et al., 2018)" is quite misleading because 40% of the 3,535 subjects in Meng et al. were already included in Steenland et al. who had reported "Restricting to studies where blood was sampled from mothers early in the pregnancy or shortly before conception (5,393 births), we found little association of PFOA with birthweight (−3.3 g [−9.6, 3.0]). In studies where blood was sampled late in the pregnancy (7563 pregnancies), lower birthweight was associated with higher PFOA (−17.8 [−25.0, −10.6])." In essence, only 60% of the Meng et al. 2018 paper were actually "new" data that had not been considered by Steenland et al. 2018. EFSA should, therefore, correct their misinterpretations of these two studies. EFSA should also acknowledge in their summary, besides Johnson et al. (2014) and Negri et al. (2017) in lines 4109-4117, the conclusions in the meta-analyses conducted by Verner et al. (2015), that also included Fei et al. (2007) in their meta-analyses of PFOS and PFOA, as well as their simulated PBPK models, that confirmed a lack of reduced birthweight associations when PFOS or PFOA are measured in the maternal first trimester.	samples is that exposure is higher compared to more recent studies and samples are taken early in pregnancy. On the other hand, the CONTAM Panel acknowledge the conclusion from the Steenland analyses, and mention possible confounding by maternal physiology and the evidence is weighted accordingly in the conclusion Regarding the Steenland and the other meta-analyses, their results miss an important aspect which is the exposure level. Most meta-analyses today take this into consideration using does-response meta-analyses. Taking an average over studies with higher and lower exposure to produce a combined effect estimate can be misleading.

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The 3M Company	185	3.3.4.4.2 Vaccination response	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 119-125: Excluding Abraham et al. (2020) due to lack of providing confidence intervals, 11 studies have examined vaccine antibody response with exposure to PFOS and PFOA (Table 3in the full comment to this submission). Percent change to tetanus antibody or diphtheria antibody per increase in PFOS or PFOA have been the outcomes most reported. As noted in Table 3 in the full comment to this submission, only one of these 11 studies reported results on Hib (Granum et al. 2013). In bivariate analyses of 50 children, they reported no statistically significant associations with reduced antibody levels to Hib, tetanus, or measles and exposures to PFOS, PFHxS, PFOA, or PFNA. Granum et al. did report statistically significant exposure-related associations with rubella in bivariate and multivariate analyses. Based on the forest plots there is inconsistent evidence to suggest an association with reduced tetanus antibody response for either PFOS or PFOA (see Figure 1A and Figure 1B in the full comment to this submission). There is imprecise evidence suggestive of an association between decreased diphtheria antibody response and increased serum concentrations of PFOS or PFOA (Figure 2A and Figure 2B). Commercially available vaccines differ depending on the nature of the vaccine antigen. Each vaccine type elicits an immune response through various molecular and cellular mechanisms. All vaccines contain various excipients including adjuvants to improve the antibody response, preservatives, stabilizers, and vehicles for delivering the vaccine which may differ substantially depending on the vaccine (Baxter, 2007). Given the minimum evidence of increased infectious disease susceptibility for FPOS (as shown in Table 3 in the full comment to this submission), it is questionable whether the	The provided plots graphically represent a subset of the results of these 6 studies. Of note, SDs for the Abraham et al. 2020 study are reported for the quintiles in Appendix K of the Opinion. We caution against considering either any formal evidence synthesis attempt or even a visual interpretation of this evidence base as presented; different study designs, overlapping populations and differing follow up periods, among other parameters, contribute to considerable underlying heterogeneity between studies which cannot guarantee the validity of any overall conclusion. Assuming that your hypothesis on interference with the ELISA assay is correct, it is unclear why such binding would lead to 'imprecise decrease in antibody response'? A consistent decrease

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			imprecise decreases in antibody response, as seen in Figures 2A and 2B (in the full comment to this submission) for diphtheria, are clinically relevant. It is worth noting that PFOS and PFOA have high degrees of binding affinity with serum albumin proteins, therefore, there might be a potential interference between these compounds and the ELISA assay components used to determine serum antibody titers.	would seem more logical. Furthermore, a decrease in antibody concentrations was also observed in animal studies, focussing on PFCs rather than antibody titres in serum. The association with infections in humans is somewhat consistent but given the methodological challenges of these types of studies the evidence is weaker compared to decreased antibody response. Increased infections for PFOS have also been reported in one animal study. This would not be consistent with your proposed hypothesis.
The 3M Company	186	3.3.4.4.3 Clinical Infections	Although we have broken out our text by section as provided for by the EFSA portal, because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 125-126: Epidemiological data do not support reduced resistance to infection In section 3.3.4.4.3, EFSA cited a total of 9 papers with their opinion of mixed results regarding an association with infection and PFAS (cf. page 125 of the draft scientific opinion). It is unclear why EFSA (2020) did not cite Abraham et al. (the critical effect study used to calculate the TWI) who did not find any association with PFOA or PFOS related to parentally reported infections with these 101 one-year children. The lack of associations with PFOA or PFOS included total number of infections, number of infections with fever, 3-day-fever experience, number of pneumonia diagnoses, number of diarrhea diagnoses, and varicella experience. Despite this omission of Abraham et al., EFSA concluded that there is some evidence to suggest that "exposures to PFASs are associated with increased propensity of infections but more studies with objective measures of infections (not self-reports are needed)." (cf. page 126 of the draft scientific opinion). In fact, as shown below in Table 2, an equally valid conclusion could have been reached by EFSA that there is insufficient evidence to	The Abraham study is poorly designed to look at infections in the children. Most of the children had been breastfed for around 6 to 12 months and during that time period they are protected by maternal antibodies. Thus, these results were evaluated but you are correct in that they should have been mentioned. This has been revised (see Section 3.3.4.4.3). We disagree with the approach of counting studies as either + or – without further considerations. Looking at declining trends in PFOS and PFOA and number of diphtheria cases is interesting, but there are several other much stronger factors at play that have to be considered including temporal changes in vaccination coverage and other factors that may influence rate of infections. Strong changes in risk factors such as smoking or alcohol consumption are often not translated into changes in health indexes based on crude summary data which are an average over many different processes. Otherwise the CONTAM Panel are aware of the NTP and Australia Health Expert Panel conclusions and others, but this

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			lead to the suggestion of an increased propensity of associations with infections and exposure for PFOS or PFOA. As shown in Figure 3 in the full comment to this submission, an analysis of the WHO time series database between 1995 and 2015 demonstrated a counterintuitive observation. In this analysis of diphtheria, an increased number of diphtheria cases were reported in 10 Western European during the production phase-out of PFOS and PFOA that began in 2000 that resulted in the declining trend in PFOS and PFOA serum concentrations in Western Europe. We speculate whether the increase in diphtheria cases in Germany and France shown in Figure 3 in the full comment to this submission might be attributable, at least in part, to lower vaccination prevalence in the increasing immigrant populations in these two countries. Reported vaccination rates were fairly constant and the 'herd health' effect needs to be considered. Nevertheless, there is no suggestive evidence that a decline in serum PFOS and PFOA concentrations in the general population that occurred between 2000 - 2015 was associated with a decline in diphtheria cases in these 10 countries. A similar analysis of the WHO time series database was attempted with reported tetanus cases, but Germany did not report tetanus data after 2000, so the analysis was considered uninterpretable. Others have weighed in on the question of risk of infection with exposure to perfluoroalkyls: National Toxicology Program (2016) Although the 2016 NTP conclusion that "exposure to PFOA or PFOS is presumed to be an immune hazard to humans" it also concluded that there was low confidence it was associated with increased incidence of infectious disease. Australia Expert Health Panel (2018):	assessment takes more recent studies into consideration not covered by those opinions.

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			"The strongest evidence for a link between PFAS and clinically important immunological effects is for impaired vaccine response. However, the human dose-response/threshold for potential immune effects is very poorly characterized, and the overall human evidence is weak." Food Standards Australia New Zealand, FSANZ (2016): A literature review commissioned by FSANZ concluded that "there are both positive and negative studies showing associations for increasing PFOS and PFOA concentrations to compromise antibody production in humans. However, to date there is no convincing evidence for increased incidence of infective disease associated with PFOS or PFOA effects on human immune function". Health Canada (2017a): "Studies in environmentally-exposed populations have identified associations between PFOS levels and decreased antibodies against various illnesses, but the influence of PFOS exposure on clinical immunosuppression (i.e., incidence of illnesses) appears to be more tenuous." Health Canada further commented that "a low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear." Health Canada reached similar conclusions regarding PFOA (Health Canada, 2017b) National Institute for Public Health and the Environment (RIVM, 2016): RIVM concluded that "associations have been found between exposure to PFOA and a decreased vaccination response", but the "evidence is unclear".	

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The 3M Company	187	3.3.4.6 Metabolic outcomes	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Additional comments re Li et al. (2020): EFSA should be aware of a very recent study that was just published that suggested a causal association between cholesterol and PFOS and PFHxS by Li et al. (2020). Li et al. (2020) studied associations between the perfluoroalkyls PFOS and PFHxS (and to a lesser degree PFOA) and serum lipids in Ronneby, Sweden, where one of two waterworks had been contaminated from aqueous film forming foams (AFFF). The original exposure occurred between the mid-1980s and cessation of exposure that occurred in 2013 through GAC filter installation. Three populations were reported: control (N =130) in a neighboring community that had not been exposed; 2) a recently exposed population (N = 1160) who lived in Ronneby defined as anytime between 2005 to 2013; and 3) a non-recent/uncertain exposure group of N = 655 who lived in the contaminated after works distribution area in Ronneby before 2005 but not after as well as participants who lived in the non-contaminated waterworks area in Ronneby anytime between 1985 to 2013. All participants were between 20 – 60 years of age. Median serum concentrations (ng/mL) were for the control, non-recent/uncertain, and recently exposure groups were, respectively (ng/mL): PFOS 4.8, 45, 240; PFHxS 0.98 40, 210; and PFOA 1.6, 3.5, 13. Comparing the control to total (combined) exposure group revealed a significantly increase in total and LDL cholesterol but not HDL, triglycerides or the total/HDL ratio. Analyzed each separately, the strongest positive lipid associations were reported for the recently exposed. Even among the controls there were modest significant associations with PFOS and PFHxS. Similar to Steenland et al. 2009, decile	The CONTAM Panel agrees that the study by Li et al. (2020), which was published after the deadline for literature inclusion, is relevant in the assessment of causality of the associations between exposure to PFASs and increased serum cholesterol. We have added to the opinion an explanation regarding the potential confounding by variability of intestinal reabsorption of PFASs and bile acids, and in connection with this also the study by Li et al. is mentioned. The CONTAM Panel agrees that this study has limitations.

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			analyses suggested the strongest associations (slopes) were observed at the lowest concentrations (up to about the 40th percentile). The recently exposed group had the highest odds ratio for high cholesterol. Li et al. conclude their findings provided additional evidence of a causal association between PFAS and serum lipids, including PFHxS, as is not necessarily confounded by the possibility of reabsorption of bile acids with the measurement of serum PFAS as some of their analyses were by categories of exposure not measurement. However, they acknowledged important limitations including (again) a cross-sectional study design, disparate socioeconomic (SES) differences between the exposed and control populations (the latter having higher SES), the lack of information on cholesterol lowering medications, and unknown dietary habits of the population. Also, the preference by the authors to cite literature that supported their position of a causal association was present. For example, they wrote humans may be less active than the rodent to the lipid lowering effects of PPARα but neither the Pouwer et al. nor Convertino et al. studies were mentioned which suggest hypolipidemic associations are likely to occur at very high (i.e., toxicological) perfluoroalkyl concentrations. Li et al. also cited a paper that had promoted the idea of a "hypercholesterolemic environment" with PFOA through its effect on the expression of genes involved in human cholesterol transport and metabolism (Fletcher et al., 2013). However, they did not to cite the evidence presented against this hypothesis by Vanden Heuval (2013).	
The 3M Company	188	3.3.4.6.1 Blood lipids	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 127-128, lines 4684-4704: EFSA's critique of the Convertino et al. study obfuscates the critical points of this study, writing "the CONTAM Panel noted that this report [Convertino et al.] cannot shed additional light on effects in humans at the much lower PFOA intake	The CONTAM Panel agree that the study by Convertino et al. (2018) suggests that very high doses of PFOA can lower serum cholesterol. The study is interesting but we note that the 3M company seems to agree on the comment in the Opinion about the study in relation to effects in humans at the much lower PFOA intake levels occurring from normal diet or contaminated drinking water. Regarding the serum PFOA concentrations after six weeks, Figure 1 in the Convertino paper is small and has $\log_{10}$ levels on

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			levels occurring from normal diet or contaminated drinking water." Of course, it cannot. Convertino et al. was never designed to do this; nor did it suggest otherwise. The Convertino et al study does, however, unequivocally show that at very high dosages of PFOA administered for 6 weeks in these human subjects resulted in decreased serum total cholesterol and LDL while HDL remain unchanged. The Convertino et al. report reviews the clinical chemistry results from a phase 1 dose escalation trial conducted in Scotland that assessed the chemotherapeutic potential of the ammonium salt of PFOA. EFSA criticizes the lack of a control group that hinders both the study's internal and external validity. This EFSA criticism lacks merit. Each subject provided their own baseline evaluation one week before the first dose was administered. As stated in Convertino et al., "for each subject, pre-treatment evaluations included a full medical history tumor evaluation, chest X-ray and 12-lead ECG, full blood count and coagulation screen, biochemical provide including urea, electrolytes, alanine transaminase (AL), aspartate transaminase (AST), alkaline phosphatase, bilirubin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, albumin, calcium, urea, uric acid, thyroid stimulating hormone (TSH), free thyroid hormone (fT4), blood glucose, urinalysis, and a physical examination." EFSA questions the non-dietary administration of PFOA in this study where gelatin capsules were administered. This is a reasonable scientific question as it was a bolus administration. However, dose was not the primary metric of exposure; internal concentrations were. This is essentially no different than EFSA accepting the daily gavage administration of PFOS in immunotoxicity studies of mice and rats. Internal serum concentrations were considered in the exposure assessment of these animals. Convertino et al. presented strong evidence of a lipid lowering effect in the phase 1 trial subjects at the very high	the Y axis. The CONTAM Panel are grateful that 3M provided the correct data for the minimum serum PFOA at 6 weeks and changed it in the Opinion. Finally, The CONTAM Panel agree that if there is indeed a causal effect between low concentrations of PFAS and higher cholesterol levels in humans, then studies on the mode of action for this are warranted.

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			dosages of administered PFOA. EFSA describes this as a 'slight' reduction in cholesterol, but it it is not slight whether analyzed and shown as linear (generalized estimating equations - GEE) or non-linear (probability density function) analyses. Based on the GEE analyses, between 420 and 566 µM PFOA resulted in a ~ 0.5 to 0.7 mmol/L reduction in cholesterol (~20 mg/dl – 25 mg/dl). Between ~ 708 to 870 µM PFOA there was a 0.8 to 1.0 mmol/L reduction in cholesterol (~30 – 40 mg/dL). EFSA did not cite the lack of changes in HDL or in liver enzymes in this six-week study. EFSA (2020) wrote serum PFOA concentrations were about 10,000 – 150,000 ng/mL after the first dose which is a fair approximation. EFSA then incorrectly stated the concentrations ranged between 150,000 – 500,000 ng/mL after six weeks. Based on the PK/PD modeling (shown in Figure 1 of Convertino et al.), as well as the data themselves, a 50 mg dose of PFOA taken weekly by the "average" 75 kg subject (see Table S1) resulted in a dose of approximately 0.67 mg/kg/week and predicted and actual PFOA concentrations of approximately 60,000 ng/mL, not 150,000 ng/mL. Regardless, these are much higher concentrations of PFOA in the general or environmentally exposed populations and exceeds those found in the upper range found in occupational studies (Olsen et al. 2000). EFSA also criticizes the Convertino et al. study for its small sample size of 49 subjects. This longitudinal study lasted 7 weeks (includes the first week of baseline measurements). Yet EFSA did not describe as "small" the critical effects study they chose to calculate their TDI, i.e., the Abraham et al. cross-sectional study of 101 infants. It is the combination of the findings from Convertino et al. and Pouwer et al. (see section3.3.5.1) , under their study conditions reported, that strongly suggest that at toxicological dosages of PFOA that yield very high serum concentrations of PFOA, a decrease in total cholesterol and LDL cholesterol is likely a causal effect. Understanding of	

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			the findings from these studies allows for those who opine there is a causal effect between low concentration of PFOA and higher cholesterol levels in humans to direct their resources on the mode of action for this hypothesis, let alone a mechanism of action. Continued publication of cross-sectional epidemiologic studies at low concentrations of PFOA are unlikely to "shed additional light."	
The 3M Company	189	3.3.5. Mode of action	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 144, lines 5475-5480. We agree with EFSA's very limited conclusion on the mode of action of immunotoxicity which states "A clear mode of action of immunotoxicity by PFOS and PFOA has not been established. Just anectdotal data are available that may offer some leads for further investigation, but do as yet not offer a clear mechanism along with PFASs influence the immune system. Information on immunotoxicty by PFAS other than PFOS and PFOA and whether such effects on the immune system might be brought about by similar mechanisms as PFOS and POFOA is even more limited." Pages 127-129, lines 4679-4753: EFSA (2018) used increased serum cholesterol outcomes from cross-sectional epidemiologic studies to derive a provisional TDI for PFOA and PFOS. EFSA (2020) now considers the uncertainty regarding causality to be larger than it did in 2018 primarily due to a hypothesis of enterohepatic cycling of both PFASs and bile acids which the latter could affect cholesterol. In fact, this uncertainty is even likely larger than what EFSA (2020) acknowledges based on five studies (four of which were not cited by EFSA). 3M's comments are comments on 2 epidemiologic studies related to serum cholesterol and 3 animal toxicological studies with exposure to PFAS conducted on a genetically engineered mouse model that mimics human lipoprotein metabolism, on cynomolgus monkeys, and a small (n = 20) study on mice examining bile acids and PFAS.	The CONTAM Panel agrees with this comment and feels it has adequately expressed this in the Opinion. Yes, the CONTAM Panel now considers the uncertainty regarding causality to be larger than it did in 2018 primarily due to a hypothesis of enterohepatic cycling of both PFASs and bile acids which the latter could affect cholesterol. The text on this issue in the opinion has now been expanded.

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			Please see our additional comments in sections 3.3.5.1 and 3.3.5.2.	
The 3M Company	190	3.3.5.1. Receptor mediated effects (MoA)	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 149, lines 5670-5671: As EFSA notes, one current leading non-causal hypothesis to explain the association between general population or environmental levels of PFOA (or PFOS) and increased cholesterol may be due to their enterohepatic circulation. Significant associations between plasma levels of PFOA and PFOS concentrations of 1.42 and 4.2 ng/mL, respectively, and bile acid levels have been reported by Salihović et al. (2019) in a "small" study of 20 subjects. Perfluoralkyls are known to downregulate CYP7A1 which is the rate-limiting step in the metabolism of cholesterol in the hepatocyte to primary bile acids. The primary bile acids are conjugated in the hepatocctyes and then both perfluoralkyls and these conjugated bile acids enter the enterohepatic circulation where the bile acids are unconjugated into bile salts by the gut microbiome. In the ileum both perfluoroalkyls and bile acids are reabsorbed into the portal venous circulated and actively transported back into hepatocytes by sodium-taurocholate co-transporting polypeptide (NTCP). Hence, the reported cross-sectional epidemiological associations between higher cholesterol levels observed in humans with lower levels of PFOA may be a consequence of this potential mode of action.	The CONTAM Panel agree that the study by Salihovic et al. (2020) is interesting, showing methods of simultaneously analysing PFAS and a large number of bile acids in serum. In the small 'proof-of-concept' study many associations between PFAS and bile acids were found, both in the positive and negative direction. This study has now been included in the additional text in the opinion on the potential confounding discussed above.
			It is unfortunate that EFSA decided not to include in their review the Pouwer et al. (2019) toxicological study who used	The impact of PFAS on blood cholesterol level and the underlying MoA are not the focus of the current Opinion. The

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			a genetically engineered APOE*3-Lieden.CETP mouse model that mimics human lipoprotein metabolism. This study confirmed the lipid lowering high dose PFOA findings from the phase 1 clinical trial in humans (Convertino et al.). This mouse model is designed to assess cholesterol ester transfer protein (CETP) expression and a delayed apoB clearance. CETP is responsible in both humans and in this mouse model for the transfer of cholesterol ester from HDL to the apoB-containing lipoproteins in exchange for triglyceride. In three different experiments lasting 4 to 6 weeks, Pouwer et al. fed these mice a Western-type diet that had four doses of PFOA (control, 10 ng/g/d PFOA, 300 ng/g/d, and 30,000 ng/g/d PFOA which resulted in plasma PFOA concentrations of approximately < 1.0 ng/mL, 50 ng/mL, 1500 ng/mL, and 90,000 – 144,000 ng/mL, respectively. In humans these concentrations reflect environmental, occupational, and toxicological exposures with the latter concentration similar to Convertino et al. Statistically significant results were observed only at the highest dose and involved decreased total cholesterol, non-HDL cholesterol, triglycerides, and elevated HDL-cholesterol. Also reported at this high dose group were increased liver weight and elevated ALT. The plasma lipid change at the high dose was explained through a decrease in very low-density lipoprotein(VLDL) production and increased VLDL clearance by the liver via increased lipoprotein lipase activity. The increase in HDL was mediated by a decrease CETP and changes in protein expression involving HDL metabolism. This APOE*3-Leiden .CETP mouse model has considerably higher concentrations of CETP than what is found in humans.	CONTAM Panel acknowledges that further research on this issue in human relevant models is required as stated also in the recommendations of the current opinion.
The 3M Company	191	3.3.5.1.3 Thyroid hormone levels and function (MoA)	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 138-140: In laboratory animal studies, thyroid pathology is considered the gold standard. If it is not feasible, the primary clinical indicator for diagnosing thyroid function is TSH, which is	As mentioned in the MoA, it is unclear why the strong decrease in levels of TT4 and TT3 do not result in a clear increase in TSH and effects on the thyroid gland. It is clear from Chang et al.

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			essential in maintaining the regulatory functions in the hypothalamus-pituitary-thyroid (HPT) axis. If there is a need for supplementary verification, it would be appropriate to measure free thyroxine (FT4), the metabolicaly-active hormone, in conjunction with TSH. It is inappropriate to use serum TT4 as a diagnostic index because it is a measurement of bound (inactive) hormone (Mendel et al., 1986; Oppenheimer et al., 1995; Refetoff et al., 1970). Studies in laboratory animals have reported that PFOS and other PFAS treatment may impact serum thyroid hormones. Using studies with PFOS as an example, serum total thyroxine (TT4) was decreased without a concomitant compensatory increase in TSH (Lau et al., 2003; Luebker et al., 2005; Seacat et al., 2002; Thibodeaux et al., 2003). This condition is usually referred to as hypothyroxinemia, which means inactive protein-bound thyroxines are being displaced from binding proteins (due to competition with PFOS for binding proteins) without altering overall thyroid hormone homeostasis. Hypothyroxinemia is commonly observed with people taking aspirin, heparin, or free fatty acids (Koulouri et al., 2013). In rodents, hypothyroxinemia is prone to occur due to their susceptibility to hepatic nuclear receptor activation. Upon activation of PPARalpha and CAR/PXR (like many PFAS do), the increased hepatic metabolic enzyme activities are often accompanied by increased conjugation and elimination of displaced thyroxines which ultimately lead to a net loss of thyroxine. However, the loss of thyroxine alone is not a disease diagnosis because the body has a reservoir of inactive (protein-bound) thyroxine and only very little of free (unbound) thyroxine is needed to maintain normal thyroid homeostatic functions. In the case of PFAS, the assays used to measure serum thyroid hormones are sensitive to the presence of compounds that can interfere and compete with thyroxine	(2007) that the decrease in the levels of free T4 may be an artefact and that the absence of a decrease in fT4 levels may explain the lack of response. The CONTAM Panel added a recommendation that this should be further investigated, including a possible relation with neurodevelopmental effects. However, effects on thyroid hormone levels were not used as the critical endpoint in this Opinion. It is currently unclear if this activation of nuclear receptors is only observed in rodents. Also in humans PPARα plays an important role and similar is true for CAR/PXR. There may be a difference in the enzymes involved in conjugation of thyroid hormones.

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			for protein bindings. This interference can negatively bias the free T4 results when conventional analog methods are used, as is the case with PFBS and other PFAS such as PFBA and PFOS (Chang et al. 2007 Toxicology 234 21-33; Weiss et al. 2009 Toxicol Sci 109 206-216; Butenhoff et al. 2012 Reprod Toxicol 33 513-530). Therefore, one must measure free T4 by equilibrium dialysis-based methods when there are high levels of PFAS present in the serum. Due to the misinformation and incorrect interpretation on thyroid-related parameters, studies with PFOS have highlighted the need to properly assess thyroid hormone function (Chang et al., 2017; Chang et al., 2009; Chang et al., 2008; Chang et al., 2007). Studies with PFOS in rats and monkeys did not show any effect on serum TSH and/or thyroid pathology when available (Chang et al., 2017; Chang et al., 2009; Chang et al., 2008; Chang et al., 2007; Lau et al., 2003; Luebker et al., 2005; Seacat et al., 2002; Thibodeaux et al., 2003). The observation of hypothyroxinemia corresponded to the increased hepatic gene transcripts reflecting increased metabolic turnover of thyroxine. The deiodinases, when evaluated, were not affected. Page 139, lines 5199-5201: The NTP 28-day with PFBS (identified as NTP 2019a) reported decreased serum total T4, total T3, and free T4 in rats at the end of 28 days dosing, but these three endpoints alone did not provide adequate (clinical) evidence to suggest that thyroid was being affected. First, NTP study did not sufficiently recognize the sensitivity of the assays used to measure serum thyroid hormones to the presence of compounds, such as PFBS, that can interfere and compete with thyroxine for protein bindings. NTP did not measure free T4 by equilibrium dialysis-based methods, as acknowledged in its report. Furthermore, total T4 and total T3 represent primarily biologically inactive T4 and T3. Thus, the total T4 and T3 and the analog free T4 do not	

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			provide sufficient or definite answers as to thyroid effects. Thyroid histology in laboratory animals should be used to determine whether there was a thyroid effect, and it was normal as reported in the NTP study, as well as in other subchronic studies (28-day and 90-day) in rats with PFBS (3M 2001; Lieder et al. 2009 Toxicology 255 45-52). Second, the serum TSH levels are the primary diagnostic indicator for thyroid hormone status. Since there were normal TSH levels and normal thyroid histology in these same rats, this suggested that overall thyroid hormone status in these rats was normal.	
The 3M Company	192	3.4.1 Critical effects	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 122, lines 4415-4442: In the current draft scientific opinion, EFSA detailed PFOA PBPK modelling results from the Abraham et al. (2020) study but did not provide any individual analyses for the other three compounds. While EFSA acknowledged and stated that "associations for PFOS, PFHxS and PFNA were not significant", these four compounds were summed together as a mixture by EFSA for the subsequent statistical analyses. This decision is not justified when EFSA stated on page 110 in their review of the supporting toxicological data that PFOS is more of the compound of toxicological interest because of its immunotoxicity.	The CONTAM Panel performed PBPK modelling on both PFOA and PFOS. See above on the use of data on the mixture rather than only PFOA from this study.
			Given EFSA had reached such a conclusion toxicologically about PFOS, it is unclear why EFSA did not provide detailed PBPK modelling results for PFOS based on the Abraham et al. (2020) study. EFSA had privileged communication with Abraham et al. for undoubtedly many months, before the public ever had a chance to read the Abraham et al. study. [Note: Abraham et al. (2020) did not appear in PubMed until March 30, 2020, two months after release of the draft EFSA	It is correct that the CONTAM Panel became aware of the study from Abraham et al. before it was published but the CONTAM Panel has used data from unpublished studies before (often provided by industry). When publishing the Opinion, the CONTAM Panel makes sure that all these data are made available to the public. For the same reason, the study by Abraham et al. was well described in the Opinion. It should also be stressed that calculations on the data were also provided by

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			(2020) report and only 3 weeks before public comments must be submitted to EFSA.] Within the data reported in Abraham et al. (2020), most of the detailed analyses focused on PFOA as that was the only perfluoroalkyl that showed statistically significantly associations. There was no comparable Figure 1 for PFOS as there was for PFOA. Table 3 did provide correlations of adjusted levels with PFOS but the remaining data displayed in Table 3 were about PFOA. Abraham et al. described the effect size in the mean reduction in antibody response when comparing the highest to the lowest quintile of PFOA exposures but such information was not found in the text or supplemental material for PFOS, PFHxS or PFNA. While Table 4 provided side-by-side data for PFOA and PFOS with the biological parameters measured, only PFOA data were provided in Figure 3 a-c, Table S2, and Figure S2. This may be the prerogative of Abraham et al. to limit their data presentation or analyses to PFOA but not PFOS, however, it cannot be the prerogative of EFSA to do the same. Because EFSA requested the actual data on the four perfluoroalkyls from Abraham et al., it behooved EFSA to analyze and present the data for each individual compound separately, especially for PFOS, given the above immunotoxicology reasons (cf. page 110 of the draft scientific opinion). Neither Abraham et al. nor EFSA provided these individual data for effect size mean reduction in antibody response from low to high quintiles and deciles for PFOS, PFHxS, or PFNA. EFSA should also provide the fitted knee functions for PFOS similar to Figure 3 (a – c) that Abraham et al. had reported for PFOA, as well as provide those for PFHxS and PFNA. A NOAEC as a point of departure was derived by EFSA via various statistical analyses based on the observational data reported in Abraham et al. (2020). 3M retained an independent statistics and modeling expert (Mr. Bruce Allen) to review EFSA's data analysis and Mr. Allen concluded that there are several technical issues with EFSA's statistical approach. The resulting faulty decisions and computations have led to a flawed NOAEC of 31.9 ng/mL (see Mr. Allen's	the authors and that the CONTAM Panel did not obtain the data on individual infants in the study.

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			report attached in Appendix A of 3M's written submission.). Mr. Allen's review is attached to this submission and discussed further in 3M's comments on Appendix K.	
The 3M Company	193	3.4.2 Mixture Approach	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 150-154: In its draft scientific opinion, PFOA, PFOS, PFHxS and PFNA were summed together as a mixture by EFSA for its derivation of the TWI. Based on the general population biomonitoring data represented as measured serum concentrations, it is evident that humans are exposed to multiple perfluoroalkyls. Due to limited hazard data available, there have been some efforts to combine these multiple exposures as a sum for the purpose of risk assessment, however, this approach has not been fully developed nor validated for perfluoroalkyls. Applying the critical concept of Toxic Equivalency Factors (TEFs) that was developed for dioxin-like compounds, Scialli et al. (2007) and Peters and Gonzalez (2011) independently evaluated the scientific feasibility of combining perfluoroalkyl exposures for risk assessment. Scialli et al. (2007) reviewed similar same-species studies performed with different perfluoroalkyls and they found large discordance in endpoints measured for PFOS, PFOA, PFBS, and PFDA. Peters and Gonzalez (2011) also concluded that perfluoroalkyl exposure should not be combined based on the following observations: (1) lack of conclusive evidence demonstrating that a single receptor is required to mediate the toxicities of perfluoroalkyl chemicals; (2) the potential influence of species differences in the response to PPARα ligands that would significantly limit this approach; (3) inconsistent toxicities observed with different perfluoroalkyl chemicals; and (4) a limited toxicological database for a number of perfluoroalkyls	For mixture approach, see above. Considering half-lives, ideally this is taken into account when deriving relative potency factors. However, this is not common practice and a qualitative approach on combining e.g. persistent congeners is more common (as in the case of dioxins). More general, there is structural similarity for several PFASs and future studies should reveal whether it is correct to include them in the HBGV. At this stage the CONTAM Panel decided to limit this to the four PFASs that are most commonly detected in human serum.

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			chemicals (e.g., perfluorinated sulfonamide polymers and perfluorinated sulfonamide-based phosphate fluorosurfactants). Therefore, the available scientific data do not support EFSA's current approach to combining perfluoroalkyl exposures in its derivation of a TWI. Page 162, lines 6114-6126: In its derivation for a combined TWI, EFSA had exerted and assigned equal (toxicity) potency for PFOA, PFOS, PFHxS, and PFNA. This was based on the following rationale: • these four PFASs contribute most to the levels observed in human serum; • these four PFASs share toxicokinetic properties and show similar accumulation and long half-lives in humans; and • these compounds in general show the same effects when studied in animals. The available data for these four perfluoroalkyls contradicted greatly with the assertions made by EFSA. Qualitatively, it is true that these four perfluoroalkyls do have longer serum elimination half-lives in humans (see Table 1in the full comment to this submission), however, there are distinct quantitative differences for the reported half-lives. In addition, given the key hazards identified by EFSA (c.f. section3.3.3 of the draft scientific opinion), Table 1 also illustrates several differences in these categorical effects with animal data. From a specific effect such as (frank) postnatal mortality in rodent pups or even adaptive liver weight increases in animals, there are large quantitative differences depending on the compounds and doses. Therefore, it is scientifically unjustifiable for EFSA to assume they all have the same effects (because they don't). The broad conjecture to assume equal potency is not supported by data, and the resulting uncertainties (as acknowledged by EFSA) bring into question the appropriateness of this methodology by EFSA.	

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The 3M Company	194	3.4.3 Dose-response assessment	We encourage you to read our full comments, which we have uploaded as a single file. This section is a high-level summary of Appendix A in the full comment to this submission. There are several technical issues with EFSA's statistical approach which reflect faulty decision-making or implementation. These issues have resulted in the flawed No Observable Adverse Effect Concentration (NOAEC)of 31.9 ng/mL. The following points highlight these concerns: 1) The reported exposure data (i.e., serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups. 2) The determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach would be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis. 3) Albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse association between serum levels of the sum of these 4 PFASs and antibody titers against Haemophilus Influenza type b (Hib)" (cf. page 7 of the draft scientific opinion).	The number of groups can affect the value of the NOAEL but based on the quintiles and deciles this effect was not very large. However, based on several suggestions to reconsider BMD modelling, a BMD approach has been used. The BMD modelling of the individual data from the study by Abraham et al. (2020) was performed by the authors, since these data could not be provided to EFSA. The CONTAM Panel was able to reproduce the results using data extracted from the graphs provided by the authors. BMDLs were obtained that could be used for the derivation of the TWI. The CONTAM Panel does not agree that the NOAEL approach is unacceptable, but that BMD modelling is preferred. The CONTAM Panel considers that there is enough information to support a causal relationship between PFAS levels and effects on vaccination response. EFSA also asked the data provider to check the p-values and it was confirmed that these were correct for the differences between quantiles but not for the ANOVA. This was corrected.

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			4) The statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach and constrained the overall data interpretation. Furthermore, summing and applying equal potency for PFOA, PFOS, PFHxS, and PFNA is not supported by science.	For mixture approach, see above.
The 3M Company	195	3.4.3.1 Effects on the immune system	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Please see our collective comments provided about immune-related discussion.	 Noted.
The 3M Company	196	3.4.3.2 Mammary gland effects	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 248, lines 5640-5643: 3M disagrees with EFSA's assessment that mammary gland development is a robust endpoint for PFOA-related toxicity in laboratory animals. There are a number of specific technical concerns that warrant careful consideration before using mammary gland data for risk characterization. To date, there has not been a standardized or even internationally recognized method of evaluating mammary gland in laboratory rodents. The studies cited by EFSA had numerous instances of inappropriate data interpretation and most studies gave very few quantitative measures pertaining to mammary gland development. These data often built on a great deal of speculation without the corroborative reproductive and/ developmental data. They were not sufficient in supporting the phenotypic consequences such as reduction in mammary gland development with exposure to PFOA. The fact that the effects of PFOA on mammary gland development cannot be consistently described and quantified in studies (Table 4) brings into question the biological	 Risk assessment of contaminants in food is not restricted to guideline studies, and the CONTAM Panel therefore considers all studies available. Qualitative or ordinal evaluation is common in pathology and histology and is not a reason for disregarding studies. The CONTAM Panel is aware of the scientific discussions around assessment of mammary gland development in guidelines on animal testing protocols (Makris, 2011 ((EHP 119:1047-1062)), Rudel et al., 2011 (EHP 119:1053-1061)). The ordinal data on mammary gland development in the studies used to derive a LOAEL are supported by the quantitative findings (duct growth, the number of TEBs) in Macon et al. (2011), in which the developmental scores on PND 20 were in line with the quantitative measurements. Overall, the studies on mammary gland development with PFOA after perinatal exposure are well in agreement (Table F1). The CONTAM Panel noticed, however, the 0-findings in the study by Albrecht et al.

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			significance of this phenotype and its relevance to human health. Elsewhere, 3M provides additional comments on this topic and several other areas of concern that EFSA needs to address in their review of published studies (see section 3.3.3.3.2 as well as Appendix C in the full comment to this submission).	(2013) which was funded by 3M. However, the study reported a low number of TEBs on PND 20 (the only time point investigated) in both PFOA treated and untreated Sv/129 mice. The CONTAM Panel is of the view that developmental changes in mammary gland as observed in mice may be relevant to human health risk assessment, irrespective of whether this is assessed quantitatively or qualitatively.
The 3M Company	197	3.6.3 Uncertainties in hazard identification and characterisation	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Page 122, lines 4415-4442: EFSA CONTAM Panel proposed a tolerable weekly intake (TWI) of 8 ng/kg/bw for the sum of four perfluoroalkyl substances: PFOA, PFOS, PFHxS, and PFNA. This value was derived based on data obtained by EFSA from data reported by Abraham et al. (2020), which was a cross-sectional study of 101 healthy 1-year old children conducted more than 20 years ago. The study reported statistically significantly negative associations between serum levels of PFOA measured in the children and their vaccine antibody responses for Hib, tetanus, and diphtheria. Abraham et al. reported no statistically significant associations between reduced antibody responses and serum levels of PFOS, PFHxS, or PFNA. Between June 1997 and May 1999, a total of 101 children living in Berlin were examined, including obtaining their blood samples, in a study whose primary purpose was to determine persistent organic pollutant exposure at the end of their breastfeeding period. These exposures included biological measurements of dioxins, PCBs, 'old' pesticides, and heavy metals. To increase study exposures in this population, a subset of this population was recruited from a dioxin hotspot. According to Abraham et al., ages of the children had to be between 341 and 369 days old and had	These issues were addressed above.

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			been breastfed maximally for 2 weeks (i.e., referred to as formula-fed children) or breastfed equivalent to a duration of exclusive breastfeeding of 4 months or longer. Children had to be considered to be healthy and vaccinated according to the recommended vaccination schedules with at least two vaccinations against diphtheria and tetanus. Specific antibodies at the time were measured via ELISA test kits for tetanus toxoid and Haemohilus influenza type b. Standard clinical chemistry assessments were also conducted as well as thyroid hormone measurements. As a consequence of the EFSA's 2018 opinion that PFOS and PFOA may result in reduced vaccination antibody titers, the principal investigator (Abraham) in 2019 decided to conduct an additional assessment of the two remaining stored plasma aliquots (stored at -80oC) to measure level of perfluoroalkyls: PFOA, PFOS, PFBS, PFHxS, PFHxA, PFNA, PFDA, PFDoDA, and ADONA. Abraham also decided to use an ELISA test kit to examine antibodies against diphtheria toxoid. Their study showed no associations with childhood infections (reported by mother). Although a standard c-reactive protein (CRP) determined samples below a level indicative of inflammatory processes, a subset of 22 children were above a more sensitive method to measure sCRP. No significant associations were observed for PFOS for Hib (r = -0.32, p = 0.66), tetanus IgG2 (r = -0.07, p = 0.52), or diphtheria, r = -0.02, p = 0.84). No significant correlations were found either for PFHxS and PFNA (data were not shown). The four perfluoroalkyls themselves were correlated with the Spearman rank correlation coefficient of 0.67 between PFOA and PFOS. The effect size differences (means for PFOA quintiles Q1 vs Q5) were -86%, -54%, and -53%, respectively, for Hib, tetanus, and diphtheria antibodies. Confidence intervals were not provided for these effect sizes. No observed adverse effect concentrations (NOAEC) were determined by fitting a 'knee' function for these three antibodies at 12.2,16.9, and 1.62 ng/mL PFOA, respectively. None of these mean effect size percentage differences were	

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			provided in the Abraham et al. paper for PFOS or the other two perfluoroalkyls.	
The 3M Company	198	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 436-437: As noted above, 3M retained an independent statistics and modeling expert (Mr. Bruce Allen) to review EFSA's data analysis and attaches his review to as Appendix A in the full comment to this submission to this submission. Provided below are four important findings from Mr. Allen's review on EFSA's data selection process: First, the reported exposure data (i.e., serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups. Second, the determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach will be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis. Third, albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse	Most issues are addressed above. Considering the PBPK modelling, The CONTAM Panel used the model developed by Loccisano et al. (2011) as the basis with only minor modifications related to growth and to human milk for exposure of infants. More information is provided now on the origin of the parameters applied, as well as a sensitivity analysis. The modifications should have little impact on the model itself, although it does not take into account changes in e.g. the relative proportion of different tissues and organs in children and pregnant women. The CONTAM Panel evaluated the model for pregnancy and lactation but this model was only available in ACSL which is no longer supported by the provider. Furthermore, the question is whether this would have resulted in a different outcome that would already be covered by the uncertainties in the risk assessment.

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			association between serum levels of the sum of these 4 PFASs and antibody titers against Haemophilus influenza type b (Hib)" (cf. page 7). Lastly, the statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach. Use of the summation approach constrained the overall data interpretation. For all of the reasons listed above, the derived NOAEC of 31.9 ng/mL is not supported scientifically. Page 162 (comments for section 3.6.4.3, PBPK modelling) For uncertainties in PBPK modelling, please refer to Appendix M, which is based on comments prepared by Dr. Anne Loccisano (at 3M's request) to assist EFSA in its current effort with PBPK modelling. Dr. Loccisano is the principal investigator who developed a series of PBPK models for the human, primate, and rat which EFSA adopted to conduct some analyses. Funds to develop these PBPK models (Loccisano et al., 2011; Loccisano et al., 2012a; Loccisano et al., 2012b; Loccisano et al., 2013) were provided by an EPA Star grant, 3M, and DuPont.	
The 3M Company	199	Appendix K – Additional information on the study from Abraham et al. (2020)	Because many of our comments are inter-related with other aspects of the proposal, we encourage you to read our full comments, which we have uploaded as a single file. Pages 436-437: As noted above, 3M retained an independent statistics and modeling expert (Mr. Bruce Allen) to review EFSA's data analysis and attaches his review to as Appendix A in the full comment to this submission. Provided below are four important findings from Mr. Allen's review on EFSA's data selection process:	Issues are already discussed above.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			First, the reported exposure data (i.e., serum PFAS concentrations) should not have been arbitrarily assigned into groups (as quintiles and deciles). The exposure-response data are observational in nature and they cannot be considered as treatment groups. Second, the determination of the NOAEC based on NOAEL/LOAEL is unacceptable and problematic. This method is not robust and it does not account for the variability and uncertainty of the data resulting from the study design itself. A better approach will be use of benchmark dose methodology that EFSA has routinely used (including for its 2018 draft opinion on PFOS and PFOA) or even of a more sophisticated Bayesian analysis. Third, albeit inappropriate in this particular case, a dose-response analysis is generally only valid for endpoint(s) that demonstrated clear evidence of a relationship between dose and response. Not only were the analyses described in the current draft opinion inappropriate, the p-values currently presented in Table K.1 appear to be incorrect. When the dose and response data were reanalyzed, the results did not support a concentration-dependent effect on Hib titer. This contradicts EFSA's conclusion that there was an "inverse association between serum levels of the sum of these 4 PFASs and antibody titers against Haemophilus influenza type b (Hib)" (cf. page 7). Lastly, the statistical approach based on the summation of the four PFAS compounds is not justified. When analyzing the data using an appropriate method by treating each compound separately, they would not have the same coefficients. EFSA's summation approach assumes that they do. Hence summation of the four PFAS compounds is an inaccurate approach. Use of the summation approach constrained the overall data interpretation.	

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			For all of the reasons listed above, the derived NOAEC of 31.9 ng/mL is not supported scientifically.	
The 3M Company	200	Appendix M - PBPK Modelling	Page 71: Clarification on placental transfer coefficients: EFSA discusses fetal/maternal serum ratios (c.f. page 71), which are then used in the PBPK model for the placental transfer coefficients (the PT parameter in the model). EFSA reports that median values for PFHxS, PFOS, PFOA, and PFNA were 0.56, 0.36, 0.74, and 0.48, respectively, which were calculated from the ratios reported by various study authors. EFSA should be specific about which values were actually used for the compounds of interest of the serum level in the infant at birth. As EFSA has assumed PFOA and PFNA would behave similarly and PFOS and PFHxS would behave similarly and thus considered them collectively with model use, it should state if only PFOA and PFOS values were used or if the values were averaged (i.e., PFNA and PFOA and PFOS and PFHxS).	This is described in the model codes. Only PFOA and PFOS values were used.
			Page 71, lines 2267-2270: Provide justification on lactational transfer ratios used in the model: Maternal-infant transfer via breast milk was estimated using the ratio of concentrations in milk versus plasma (the RATIO parameter in the model). EFSA reports the ratio is in the range of 0.01 – 0.07 for PFHxS, PFNA, PFDA, PFUnDA, and FOSA, indicating the transfer occurs at similar levels as for PFOS (ratio of 0.01 – 0.02) but to a lesser extent than for PFOA (ratio of 0.03-0.12). Values of 0.03 and 0.015 are used for PFOA and PFOS, respectively in the model. EFSA should provide rationale for use of these values, given the reported range.	Based on the comments from stakeholders, the CONTAM Panel have now performed a sensitivity and uncertainty analysis for lactational transfer ratios. It appears that such variations have a medium influence on the serum concentration estimated at 1 year of age with the PBPK model.
			Page 71, lines 2270-2282: Specify PFAS clearance coefficients in breast milk: Mondal et al. 2014 calculated that each month, breastfeeding was associated with 1 and 2% decreases in maternal serum concentrations of PFHxS and PFNA, respectively, compared to 3% for both PFOS and PFOA. Others estimated the decline	According to the same analysis, it appears that such variations in decrease in breast milk concentrations have only a low influence on the serum concentration estimated at 1 year of age with the PBPK model.

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			in PFAS concentration in breast milk due to breastfeeding. In addition, calculations can be based on intakes, distribution volumes, breastfeeding amount, and ratios between breast milk and serum concentrations. According to EFSA, all methods various authors have used show that monthly decreases in breast milk concentrations ranged from 1.2-3.1% for PFOS and 1.3-7.7% for PFOA. Percentages reported and, for PFHxS and PFNA, varied between 1-6.7% and 1.1-2.8%, respectively. EFSA chose to use a value of 3.1% for PFOS and 7.7% for PFOA in the model (the DECLINE parameter in the model). These values should be explicitly cited (i.e., the studies that reported these conclusions). If EFSA performed its own analyses to obtain these numbers, that should be described.	
			Pages 444-456: Lack of a model schematic: Appendix M describes modifications made to a previously published PBPK model for PFOA and PFOS in adult humans (Loccisano et al., 2011). Modifications made to the code are straightforward (equations for placental transfer and breast milk transfer were added as well as an equation for growth). The added equations appear to be adequate, however, it is not clear if a single model was used to estimate maternal daily intake and child intake or if the model was modified and used separately for the child and mother. A model schematic would help clarify this.	The same models were used to estimate the maternal serum concentration at 35 years and the serum concentration at 0, 0.5, 1 and 5 years in children. The maternal daily intake (and child intake after breastfeeding) are described in the model code. The serum concentration in children (<1 years) is completely determined by maternal serum concentrations (placental transfer during pregnancy and breastfeeding).
			Page 155, lines 5874-5875: Gestational and lactational models not used: P. 155, states "The graphs [referring to Fig. 12 and 13] do not include the effect of having a child and of subsequent breastfeeding, which results in a (temporary) decline in the serum levels of the mothers." On p. 163, EFSA states the models do not take into account possible physiological changes during pregnancy, which could alter the pharmacokinetics of PFASs during this period and could influence transfer to the fetus. There are separate pregnancy and lactation models for PFOA and PFOS, both of which have been published (Loccisano et	The process by which the CONTAM Panel determined the daily intake is as follows: 1-Calculation of the serum level at delivery and during breastfeeding which leads to the $BMDL_{10}$ serum level in the child at 1 year. 2-Calculation of the exposure (mother's daily intake) in order to obtain this serum concentration at 35 years (age of pregnancy).

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			al., 2011). As EFSA states that the inclusion of pregnancy could alter the pharmacokinetics and transfer to the child, rationale for not including the pregnancy life stage when there are available models should be provided. Page 154, lines 5837-5857: Clarification on maternal intake: The process by which EFSA used the PBPK models to estimate maternal intake is not well-described. Each step in estimating maternal daily intake (for both PFOA/PFNA and PFOS/PFHxS) should be outlined. If milk concentration was estimated first and then maternal intake was estimated to give this milk concentration that should be clearly stated. Concentrations the mother was exposed to in utero and during breastfeeding also should be clearly outlined. Page 154, lines 5856-5857: Technical clarification on kinetic behaviors for PFNA and PFHxS: EFSA used a modified PBPK model to estimate maternal daily intake. Data for PFNA and PFHxS were insufficient for modeling; thus, it was assumed that these compounds bNOAEC of 31.9 ng/mL is not supported scientifically.	The models described in Appendix M allow performing these two-steps simultaneously. For children after breastfeeding, the CONTAM Panel applied a double intake compared to the mother and this should be simulated in a separate run.
The 3M Company	201	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex I of this Technical report.*	-

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Mamme No Pfas	202	Abstract	Line 31 To insert This Opinion does not refer to local situations of high PFAS contamination caused by episodes of environmental pollution from PFAS nor to workers professionally exposed to PFAS (PFAS production plants, firefighters, waxers, assigned to specific tasks in the industries using PFAS) **Reasons** The EFSA Opinion does not mention the hot-spot situations present in the European Union. However, these situations must be clearly considered separately for several reasons. From the point of view of food, they can present very high PFAS contamination values, even 2-3 orders of magnitude higher than the median values from food sampled in uncontaminated areas. Above all, in consideration of the high biopersistence of PFAS in the human organism and the very high levels of serum contamination found in European populations that have suffered the consequences of heavy industrial pollution, it should be considered the fact that even moderate daily intake can translate into a even very long prolongation of human exposure above the values considered protective. HBVG values are still characterized by high uncertainty and have already experienced reductions of 2-3 orders of magnitude over time; on the other hand, safe, effective and sustainable pharmacological and instrumental interventions capable of accelerating the body clearance of PFAS are not currently available and the only possibility that remains for exposed populations is to reduce the intake of water and food to a minimum contaminated. For example, the present opinion considers that a serum level of 12.6 ng / mL in the 35-year-old pregnant woman (referred to the sum of PFOA, PFOA, PFNA and PFHxS), represents a safety criterion for an effective response to vaccinations in infants under one year of age who are breastfed. However, these levels are reached and exceeded by the vast majority of women of childbearing ageand mothers living in the red zone. In a context of high human exposure, the reference to EFSA's TWI is completely	As stated in the response to comment number 70, the risk assessments of the CONTAM Panel cover the general population and not incidents or hot spots, unless specifically requested by the Commission. However, the derived critical serum levels and TWI will apply also to populations from such areas. The CONTAM Panel is aware that the type of PFASs in such a hot spot may also differ from the ones in the general population and a more specific assessment may be required to deal with that. Also the relative contribution of food groups and drinking water may be clearly different.

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			inappropriate and misleading, while all public health measures aimed at minimizing the overall dietary intake (drinking water and food) must be taken. https://www.researchgate.net/publication/340268724_Internal_exposure_to_perfluoroalkyl_substances_PFASs_and_biological_markers_in_101_healthy_1-year-old_children_associations_between_levels_of_perfluorooctanoic_acid_PFOA_and_vaccine_response **Table of contents** Line 556 TO INSERT Appendix M1 - PBPK modeling for populations with high exposure due to environmental pollution. **Reasons** We propose an exercise conducted with a first order compartmental pharmacokinetics model, asking the CONTAM group to explain a similar exercise with the compartmental models used for the opinion formulation. https://www.researchgate.net/publication/336698598_An_approach_to_the_esteem_of_the_health_benefits_expected_from_mitigating_the_human_exposure_to_PFOA	
Mamme No Pfas	203	Summary	Line 257 replace "no evidence for carcinogenicity of PFOS and PFOA in humans as concluded by EFSA (EFSA CONTAM Panel, 2018)" with "Limited evidence of carcinogenicity, in accordance with the conclusions of IARC, 2016 and iEFSA CONTAM Panel, 2018". Line 283 replace "Although the association with increased cholesterol was observed in a large number of studies, the CONTAM panel now considers the uncertainty regarding causality to be larger. This is primarily due to a postulated biological process around the enterohepatic cycling of both PFASs and bile acids, the latter affecting serum cholesterol levels. This should be further investigated."	The wording 'no evidence' has been modified in the Opinion. The further rewording proposals provided in this comment have been reviewed by the CONTAM Panel, however the original opinion wording is considered appropriate and will not be modified.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			With "In the previous opinion on PFOS / PFOA numerous epidemiological studies had been considered on the association between PFOS / PFOA and serum lipids (EFSA CONTAM Panel, 2018). Some of these studies had been carried out in worker cohorts but most of them came from the general population. Most consisted of cross-sectional studies, but some were also longitudinal studies. After the opinion on PFOS / PFOA some new studies have examined the association between PFASs and serum lipids. A large number of epidemiological studies provide clear epidemiological evidence of the association between exposure to PFOS, PFOA and PFNA and an increase in cholesterolemia (Line 6374). A biological enterohepatic reabsorption process of both PFASs and bile acids has been postulated, which however lacks strong experimental evidence and requires further investigation " Line 293 to Insert For the selection of the critical effect used for the definition of the TWI EFSA CONTAM Panel 2020 - deviating from the opinion of the previous EFSA CONTAM Panel 2018 - he gave his preference to a criterion that provides for the presence of the same biological effect (reduction of the immune response) considered relevant for both laboratory animals and humans. However, it should be underlined that the presence of a biological effect of clinical relevance (for example: a hypercholesterolaemia requiring pharmacological treatment) cannot be excluded where controlled studies of analytical epidemiology -carried out with different methodologies in different populations - give clear evidence of a consistent and biologically plausible association based on available knowledge. Line 390 In consideration of the important environmental, chemical, biological, toxicological, epidemiological studies that have been carried out after the review of Literature performed in	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			outsourcing for the formulation of this opinion and the high degree of uncertainty that characterizes different aspects of this evaluation, this Opinion it is to be considered provisional and will be reviewed within 6 months.	
Mamme No Pfas	204	1.3.5. Previous risk assessments	Line 1138 Insert 2018 RIVM in the References. https://www.rivm.nl/bibliotheek/rapporten/2018-0070.pdf Line 1146 Insert before the Michigan Science Advisory Workgroup's toxicity assessment, which was published subsequently, also in the References.	This RIVM (2018) reference was missed out in error from the reference section, but has now been added.
			In November 2019, EPA published a draft toxicity assessment for PFBS and HPFO-DA (GenX Chemicals), which was peer-reviewed and received comments from the public by January 2019. The proposed RfDs are 2000 ng/kg-day for PFBS (critical effect: decreased T4 in newborn mice and histological alterations in the kidney of female mice at P0) and 80 ng/kg-die for HPFO-DA (critical effect: relative increase in mouse liver weight accompanied by hypertrophy and necrosis of individual liver cells). https://www.epa.gov/sites/production/files/2018-11/documents/pfbs_public_comment_draft_toxicity_assessment_nov2018-508.pdf https://www.epa.gov/sites/production/files/2018-11/documents/genx_public_comment_draft_toxicity_assessment_nov2018-508.pdf	The EPA reports cannot be cited in the Opinion, as they were available only as drafts for public consultation review purposes.
			Line 1154 Insert The environmental protection agencies of several Member States have recognised that the control actions implemented to date have been inadequate. In particular, they have acknowledged that carrying out authorisation, control, environmental monitoring and health monitoring actions separately for each compound is technically and financially	This suggested insertion has not been added to the Opinion. The task of the CONTAM Panel was to perform a risk assessment on PFASs in food. The potential consequences and follow-up actions are the remit of risk managers.

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			infeasible and unsustainable. In order to minimise human and environmental exposure to: https://www.documentcloud.org/documents/6586418-EU-Strategy-for-PFASs-FINAL-VERSION-December-19.html **Justification** The RiMar/MITENI chemical plant, located immediately upstream of the contaminated area, started to produce and release PFAS into the environment back in 1966. (Fontana GL, 2009) https://www.poligrafo.it/trissino-nel-novecento. The factory manufactured PFOA until 2014 and APFO and PFOS until 2005; it also produced PFBS. (Girardi 2019) https://pubmed.ncbi.nlm.nih.gov/31542491-a-mortality-study-on-male-subjectsexposed-topolyfluoroalkyl-acids-with-high-internal-dose-of-perfluorooctanoic-acid/ The company manufactured GenX chemicals from 2013 to 2018, and went bankrupt shortly afterwards (November 2018). Moreover, the industrial district to the north of the red zone has a number of important manufacturing companies that use PFAS in their processes; the ARICA sewer still today collects the civil and industrial wastewater from the entire area immediately upstream of the red zone. The wastewater is discharged into surface waters and contaminates the crop and livestock sectors of a mostly agricultural territory (PDO). In 2013, the following PFAS compounds were found in the highest concentrations in the wastewater from the ARICA collector sewer: PFOA 4.7 ug/L, PFHxA 5.4 ug/L, PFPeA 6.2 ug/L and PFBS 26 ug/L. https://www.minambiente.it/sites/default/files/archivio/allegati/reach/progettoPFAS_ottobre2013.pdf	

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			At the end of 2018, concentrations at the sewer discharge point had decreased three- to four-fold; PFBS and PFOA are currently the prevalent chemicals. The discharge flow rate is estimated at about 100,000 L/day on an annual basis. https://www.arpa.veneto.it/arpav/pagine-generiche/allegati-pagine-generiche/pfas-relazioni-attivita-arpav/AGGIORNAMENTO%20RELAZIONE%20PFAS%20_ARPAV%202019_05_13.pdf A technical difficulty in analysing wastewater chemistry is the presence of macromolecules that can release PFAS. https://www.epa.gov/sites/production/files/2019-06/documents/method_8327_executive_summary.pdf https://www.epa.gov/sites/production/files/2019-06/documents/method_8327_executive_summary.pdf (ISO25101:2009) The experience of the citizens of Veneto and many other European areas exposed to chemical pollution from plants that manufacture and use PFAS is unequivocally one of exposure to 'new' chemical compounds as the 'old' ones are discontinued. For example, the Rimar-MITENI chemical plant, which operated from 1966 immediately upstream of the contaminated area, caused a serious episode of drinking water pollution from dibenzotrifluorides back in 1977, followed by environmental contamination with PFOA, PFBS and 10 other PFAS (reported in 2013) and GenX chemicals (reported in 2018).	

Stakeholder	Comment number	Chapter	Comment	EFSA response
Mamme No Pfas	205	3.1.1. Current occurrence data in food	Line 1436 Replace For EFSA, this was a reason to start an inquiry. With According to EFSA, the occurrence of unusually high levels of food contamination with PFASs, even where determined with non-validated methods, may be a reason to start an inquiry. Food and drinking water samples from areas with high environmental contamination in various regions of Italy, Germany and the Netherlands were excluded from the data on PFASs occurrence and contamination (see Line 11425).	The rewording proposals in this comment have been reviewed by the CONTAM Panel, however the original opinion wording is considered appropriate and has not been modified.
Mamme No Pfas	206	3.1.2. Analytical results	Line 1544 The LOQ cut-off of 1 ng/g (Table 4, Table 5) used by EFSA is not suitable for estimating dietary exposure: this is because the comparison of analytical contamination data expressed as LB/UB shows deviations of more than 100% for several food categories. As a benchmark, in the analysis of dioxins the difference must not exceed 40%. This deviation is then reflected in intake estimates, where the difference between LB/UB dietary exposures increases the gap. https://pubmed.ncbi.nlm.nih.gov/24279394/ https://pubmed.ncbi.nlm.nih.gov/23859821/ **Justification** Consumer associations, individual citizens and even some producers living in the red zone have ordered laboratory tests at their expense, on locally produced food, finding very high contamination levels. "I will now report on the case of one local resident. A farmer from Lonigo used mains water for cooking, while for drinking he had always bought glass-bottled water from a spring upstream of the pollution source. When he heard about PFAS pollution he felt fairly safe, since he followed a healthy lifestyle and only used mains water for cooking. However, when he received the lab test results, he found PFAS values of around 300 ng in his children's blood. He immediately thought of his crops, which were his family's main food	The issue with to high LOQs/LODs was noted and the reason for the large difference between the LB and UB exposure assessment. Using a lower cut-off would have resulted in the loss of many data, both LC and quantified levels where a method with high LOQ was used. There are no MLs for these compounds that could be used to derive guidelines on the sensitivity of methods, as is the case for dioxins. There is a recommendation to develop and use more sensitive methods.

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			source. He thus had his crops tested for PFAS concentrations. The results were astonishing: for example, his kiwi fruits contained 31 ng/g of PFAS (31,000 ng/kg). This is why we do not feel we can safely consume the food produced in our area". http://documenti.camera.it/leg18/resoconti/commissioni/stenografici/html/39/audiz2/audizione/2019/10/15/indice_stenografico.0047.html	
Mamme No Pfas	207	3.3.2.3 Levels in general European populations with serum or plasma samples collected from 2007–2008 and onwards	Line 2511 Insert, also in the References. We can expect the future time trends of PFAS concentrations to be closely linked to highly temporally and spatially variable factors, such as the type and quantity of PFAS produced and used by industry, the vulnerability of surface and groundwater bodies, the extent of crop and livestock farming activities in the areas contaminated by wastewater discharge and, last but not least, the environmental remediation actions implemented (Land, 2018). https://environmentalevidencejournal.biomedcentral.com/track/pdf/10.1186/s13750-017-0114-y In areas with intense PFAS manufacturing and use, a priority area for research is to carry out adequately representative continuous human monitoring studies, including through the organisational and technical opportunity offered by neonatal screening Line 2531 Insert in Table 16: Maximum PFOA 754.4 ng/g - (reference fo maximum: Ingelido 2018, not reported if EFSA CONTAM 2018) Line 2538 Insert in Table 17: Maximum PFOS 70.3 ng/g - (reference fo maximum: Ingelido 2018, not reported in EFSA CONTAM 2018)	Land et al., 2018, was already included in the Opinion in relation to temporal trends in the sections on Environmental fate and Biomonitoring. No further wording insertions are required. For PFOS and PFOA the values in the Tables 16 and 17 are those from the 2018 EFSA CONTAM Opinion, and are based on the available literature at that time. For the other PFASs, later studies are included.

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			https://pubmed.ncbi.nlm.nih.gov/29108835-biomonitoring-of-perfluorinated-compounds-in-adults-exposedto-contaminated-drinking-water-in-the-veneto-region-italy/ Line 2549 Insert in Table 18 and in the References. Health Surveillance Plan on 2,391 children aged 9-13 years living in an area with drinking water pollution in the Veneto Region. http://www.quotidianosanita.it/allegati/allegato7684276.pdf min median average max Line 2558 Insert in Table 19 and in the References. Health Surveillance Plan on 2,391 children aged 9-13 years living in an area with drinking water pollution in the Veneto Region. http://www.quotidianosanita.it/allegati/allegato7684276.pdf	The data included in the Tables 18 & 19 comes from peer reviewed articles which were published up to end Aug 2018, this report was published after this deadline (19 Dec. 2019) and cannot be included at this late stage.
Mamme No Pfas	208	3.3.2.4 Levels in blood from occupationally exposed adults	Line 2564 Insert The workers of a fluorochemical plant in the Veneto Region (Italy) recently affected by a serious environmental contamination incident, also showed extremely high serum PFOA levels (in 2000: median 11.9, p75 32.0, maximum 86.3 ug/mL; in 2007: median 11.6, p75 18.7, maximum 47.0 ug/mL) (Costa, 2009). https://pubmed.ncbi.nlm.nih.gov/19225424-thirty-years-of-medical-surveillance-in-perfluorooctanoic-acid-production-workers/ **Justification** A large area between the Provinces of Vicenza, Verona and Padua in the Veneto Region has been affected by an episode of drinking water and food supply chain pollution and human exposure, which has caused the largest documented episode	Olsen, 2015 (*Olsen GW, 2015. PFAS biomonitoring in higher exposed populations. In: De Wit CA (ed.). Toxicological Effects of Perfluoroalkyl and Polyfluoralkyl Substances. Series: Molecular and Integrative Toxicology, Springer International Publishing, Switzerland. 77–125*), cited and discussed in this section (and in the 2018 EFSA CONTAM Panel Opinion) summarises several studies on occupational exposure and this includes the study by Costa (2009). No further additions are required to this section of the Opinion.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			of human contamination at international level. https://bur.regione.veneto.it/BurvServices/Pubblica/Download.aspx?name=691_AllegatoA_370611.pdf&type=9&storico=False The human contamination was studied by the Istituto Superiore di Sanità (Italian National Institute of Health) by means of a sampling and biomonitoring study carried out in 2014 on 257 individuals aged 20-51 years living in the red zone, which has a total population of about 60,000 people. In 2017, the Veneto Region started a Health Surveillance Plan for the red zone, which to date has covered around 34,967 individuals aged 14-65 years and 2,391 individuals aged 9-13 years, recruited in annual cohorts. https://bur.regione.veneto.it/BurvServices/Pubblica/DettaglioDgr.aspx?id=336975 http://www.quotidianosanita.it/allegati/allegato7684276.pdf The serum contamination levels found in the workers of the RiMAR/MITENI plant are also among the highest recorded internationally. https://pubmed.ncbi.nlm.nih.gov/31542491-a-mortality-study-on-male-subjects-exposed-to-polyfluoroalkyl-acids-with-high-internal-dose-of-perfluorooctanoic-acid/ In 2017, the Veneto Region started a Health Surveillance Plan for the red zone, which to date has covered around 34,967 individuals aged 14-65 years and 2,391 individuals aged 9-13 years, recruited in annual cohorts. https://bur.regione.veneto.it/BurvServices/Pubblica/DettaglioDgr.aspx?id=336975 http://www.quotidianosanita.it/allegati/allegato7684276.pdf	
Mamme No Pfas	209	3.3.4.1 Fertility and pregnancy outcomes	Line 4090 To insert A report by the Regional Observatory on Mother and Child Health of the Veneto Region used the birth assistance certificates relating to mothers residing in the red zone. The exposure was determined in an ecological way on the basis	The section on fertility and pregnancy outcomes includes the available studies from primarily peer reviewed literature. All available studies were reviewed and information from one report or a study would not have major influence on current conclusions on pregnancy outcomes

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			of the municipality of residence of the parturient, while several known confounding factors have been adjusted individually. There has been an increase in births with low birth weight by gestational age (EMS) and with some congenital anomalies and an increase in pre-eclampsia and pregnant diabetes in mothers. http://www.quotidianosanita.it/allegati/create_pdf.php?all=590363.pdf **Reasons** Fortunately, there are not many areas in Europe and in the world characterized by a large, intense and prolonged population exposure such as that which occurred in some areas of Italy, Germany and Holland characterized by heavy industrial pollution. Scientific research must be a public health priority for the documentation of rare events such as low birth weight for gestational age (EMS). Furthermore, it is necessary to establish continuous monitoring on the exposure to current and future PFASs in the cohorts of newborns in the polluted areas, in order to be able to carry out longitudinal studies of sufficient power. https://www.iresearchgate.net/publication/23220811_Use_of_Newborn_Screening_Program_Blood_Spots_for_Exposure_Assessment_Declining_Levels_of_Perfluorinated_Compounds_in_New_York_State_Infants https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6376484/ https://pubmed.ncbi.nlm.nih.gov/30296759/	
Mamme No Pfas	210	3.3.4.5.2 Male fertility and puberty	4663 Replace In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of cross-sectional studies on semen quality and sex hormones in men were reviewed. In summary those studies did not provide support for serum PFOS/PFOA as predictors of adverse effects on semen quality or reproductive hormones. Some of these studies also examined other PFASs than PFOS and PFOA, as described in appendix J. In addition, one additional study reporting	The rewording proposals in this comment have been reviewed by the CONTAM Panel, however the original opinion wording is considered appropriate and has not been modified.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			results on PFOS and PFOA have been published after the PFOS/PFOA opinion. In summary, the available evidence is insufficient to suggest that pre- or postnatal exposures to PFASs are associated with effects on pubertal development or male fertility. with In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of cross-sectional studies on semen quality and sex hormones in men were reviewed. New reaserch has been published and the actual available epidemiological and in-vitro evidence is moderate to suggest that pre- or postnatal exposures to PFASs are associated with effects on pubertal development or male fertility. Cite Foresta C, Zuccarello D, Garolla A, Ferlin A. Role of hormones, genes, and environment in human cryptorchidism. Endocr Rev. 2008 Aug;29(5):560-80. doi: 10.1210/er.2007-0042. Epub 2008 Apr 24. PMID: 18436703. Di Nisio A, Sabovic I, Valente U, Tescari S, Rocca MS, Guidolin D, Dall'Acqua S, Acquasaliente L, Pozzi N, Plebani M, Garolla A, Foresta C. Endocrine Disruption of Androgenic Activity by Perfluoroalkyl Substances: Clinical and Experimental Evidence. J Clin Endocrinol Metab. 2019 Apr 1;104(4):1259-1271. doi: 10.1210/jc.2018-01855. PMID: 30403786. Šabović I, Cosci I, De Toni L, Ferramosca A, Stornaiuolo M, Di Nisio A, Dall'Acqua S, Garolla A, Foresta C. Perfluoro-octanoic acid impairs sperm motility through the alteration of plasma membrane. J Endocrinol Invest. 2019 Nov 27. doi: 10.1007/s40618-019-01152-0. Epub ahead of print. PMID: 31776969. Di Nisio A, Foresta C. Water and soil pollution as determinant of water and food quality/contamination and its impact on male fertility. Reprod Biol Endocrinol. 2019 Jan 6;17(1):4. doi: 10.1186/s12958-018-0449-4. PMID: 30611299; PMCID: PMC6321708.	

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Mamme No Pfas	211	3.3.4.5.3 Female fertility, menstrual cycle and puberty	Line 3762 Replace In the opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018) a number of studies on semen quality and sex hormones in men were reviewed. In summary those studies provided insufficient evidence that exposure to PFOS or PFOA is related to menstrual cycle length, menopause or puberty development. Likewise, the studies reviewed in this opinion (Appendix J) do not provide consistent associations between pre- or postnatal exposures to PFASs and female reproduction outcomes or puberty. With Previous studies on female fertility, the menstrual cycle and female puberty were reviewed in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel 2018). Since then new studies have been published. The current evidence of epidemiological research and in-vitro studies suggests moderate evidence of the association between pre- and post-natal exposures on the outcomes of puberty and female fertility Insert in the References. Di Nisio A, Rocca MS, Sabovic I, De Rocco Ponce M, Corsini C, Guidolin D, Zanon C, Acquasaliente L, Carosso AR, De Toni L, Foresta C. Perfluorooctanoic acid alters progesterone activity in human endometrial cells and induces reproductive alterations in young women. Chemosphere. 2020 Mar;242:125208. doi: 10.1016/j.chemosphere.2019.125208. Epub 2019 Oct 24. PMID: 31896193.	The rewording proposals in this comment have been reviewed by the CONTAM Panel, however the original opinion wording is considered appropriate and has not been modified.
Mamme No Pfas	212	3.3.4.6 Metabolic outcomes	Line 4750 Replace "Associations between PFOS/PFOA and cholesterol has been reviewed again after external comments to the previous opinion. Further details are presented in section 3.4. As illustrated there, the CONTAM panel now considers the uncertainty regarding causality is larger than what was stated in the previous opinion."	The rewording proposals in this comment have been reviewed by the CONTAM Panel, however the original opinion wording is considered appropriate and has not been modified.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			with "In the previous opinion on PFOS / PFOA numerous epidemiological studies had been considered on the association between PFOS / PFOA and serum lipids (EFSA CONTAM Panel, 2018). Some of these studies had been carried out in worker cohorts but most of them came from the general population. Most consisted of cross-sectional studies, but some were also extensive longitudinal studies. The association between PFOS / PFOA and hypercholesterolemia was again considered in the light of studies published after the previous scientific opinion of the CONTAM EFSA Panel of 2018. Further details are presented in section 3.4. The review of the literature relating to the effects of exposure to PFASs on plasma lipids carried out for this scientific opinion mainly considers the number of studies that show / do not show statistically significant results. This approach, however, is conditioned depending both on the number of the sample in the study and, critically, on the quality of the study protocol; a further in-depth approach based on the strength of evidence is therefore necessary. Further research may clarify the mechanism of action in humans which justifies the clear epidemiological evidence of the positive association between serum exposure to PFASs and hypercholesterolemia. A biological enterohepatic reabsorption process of both PFASs and bile acids has been postulated, which however lacks strong experimental evidence to date. " **Reasons** In the red zone, a Health Surveillance Plan has been launched since 2017 by the Veneto Region, which has so far involved around 34,967 people aged 14-65 and 2,391 people aged 9-13. https://bur.regione.veneto.it/BurvServices/Pubblica/DettaglioDgr.aspx?id=336975 http://www.quotidianosanita.it/allegati/allegato7684276.pdf	

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			- The data relating to plasma lipids - even if they give only an aggregate description of the phenomenon to date - are a source of concern, in particular for children aged 9-13, who have a limited duration of dietary exposure to animal fats - Total cholesterol 158 mg / dL,% out of the norm 12.8% - LDL cholesterol 86 mg / dL,% out of the norm 12.3% - HDL cholesterol 58 mg / dL; % out of the norm 5.0% - Triglycerides 57 mg / dL; % out of the norm 2.4% The figures for adults are, as expected, even higher	
Mamme No Pfas	213	3.3.4.8 Carcinogenicity outcomes	Line 5011 Replace "It was noted that this is in line with the conclusion from the recent IARC report on PFOA (IARC, 2016), which concluded that there was limited evidence for carcinogenicity." With 'It was noted that this is in line with the conclusions of the recent IARC report (IARC, 2016), which concluded that there is limited evidence of carcinogenicity (possibly carcinogenic to humans – group 2B), - limited evidence in humans for the carcinogenicity of PFOA. A positive association was observed for cancers of the testis and kidney - limited evidence in experimental animals for the carcinogenicity of PFOA'. Subsequently, on 30 October 2019, the report of the Advisory Group to Recommend Priorities for the IARC Monographs during 2020-2024 assigned high priority to review of the 2016 monograph, which contains the initial assessment for PFOA. https://monographs.iarc.fr/wp-content/uploads/2019/10/IARCMonographs-AGReport-Priorities_2020-2024.pdf On 12 December 2020, the NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid reported on the experimental results of perinatal	Modifications have been made to this section of the opinion, as appropriate**.**

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			exposure to PFOA of Sprague- Dawley rats, concluding that there is clear evidence of carcinogenic activity of PFOA in male rats and someevidence of carcinogenic activity in female rats. https://ntp.niehs.nih.gov/ntp/about_ntp/trpanel/2019/december/tr598draft.pdf In conclusion, the carcinogenic risk of PFOA and other PFAS is a well-established international priority'. Line 5013 replace "After the PFOS/PFOA opinion, some additional studies have been published on other PFASs. In addition, one study has been published reporting only results on PFOS and PFOA. In summary, taking into consideration the review if studies provided in the previous opinion on PFOS and PFOA (EFSA CONTAM Panel, 2018), these three additional studies provide no evidence for a link between exposure to PFASs and cancer risk." With 'Since the previous opinion on PFOS/PFOA (EFSA, 2018) several studies on other PFAs have been published. A further study with results on PFOS and PFOA only has been published. These additional studies refer to a cancer site (breast) that was not considered in the conclusions of the IARC monograph on PFOA, and do not provide additional evidence on the association between exposure to PFASs and the cancer sites identified as being of interest in the IARC monograph (kidney, testicle)'. Line 5016 Insert In 2019 a prospective study among 120 workers from the chemical plant settled upstream the most contaminated area in the Veneto Region was published as well (Girardi 2019). An excess standardized mortality ratio was found as to liver	

Stakeholder	Comment number	Chapter	Comment	EFSA response
			cancer (SMR 2.32, CI 95% 1.11-4.87), and malignant neoplasm of lymphatic and haematopoietic tissue in comparison with the general population. The mortality was increased in comparison with a cohort of workers (SMR 6.69, CI 95% 1.71-26.2 for liver cancer and SMR 3.87, CI95% 1.18-12.7 for liver cirrhosis). https://pubmed.ncbi.nlm.nih.gov/31542491-a-mortality-study-on-male-subjects-exposed-to-polyfluoroalkylacids-with-high-internal-dose-of-perfluorooctanoic-acid/ **Justification** In particular, according to the scientific evaluation of IARC 2016, 'The evidence for cancer of the testis was considered credible and unlikely to be explained by bias and confounding; however, the estimate was based on small numbers'. A case-control study based on cancer registry data suggests an increased risk of testicular cancer (6 observed vs 2.1 expected) for internal exposure to PFOA above 110 ng/mL in serum, with residential exposure and latency of at least 10 years (Vieira 2013). This may well be the case for populations exposed to high environmental pollution (see. for example, the health surveillance study carried out in the red zone: p75 for PFOA 74.9 ng/g). http://www.quotidianosanita.it/allegati/allegato7684276.pdf In 2016, a retrospective ecological study on hospital discharge records after testicular cancer surgery examined the data collected between 1997 and 2014 for males aged 15-54 years resident in the red zone, finding excess risk in the municipality of Lonigo (16 observed cases vs 8.7 expected, with a standardised incidence rate of 1.84; 95% CI 1.05- 2.98). http://www.euro.who.int/__data/assets/pdf_file/0018/340704/FINAL_pfas-report-20170530-h1200.pdf The Chair and the members of the CONTAM Panel dealing with PFASs are strongly invited to correct the error contained in the Summary (line 257).	

Stakeholder	Comment number	Chapter	Comment	EFSA response
Mamme No Pfas	214	3.4.1 Critical effects	Line 5697 Insert Of particular interest is the decrease in antibody response in children under one year of age, when the immune system is still immature and the risk of invasive diseases is very high. https://www.ncbi.nlm.nih.gov/pubmed/32227269	The addition of this text suggestion is not considered necessary.
Mamme No Pfas	215	3.6.4 Uncertainties in dose-response assessment and HBGV derivation	Line 6090 Insert The current EFSA CONTAM Panel has considered the traditional NOAEL/NOAEC approach instead of the Benchmark Dose Modelling approach, which takes into account the overall dose-response curve and which could result in identification of a significantly lower no-effect/minimal effect threshold. The current draft does not consider some uncertainty factors, since the assessment is based on human toxicity data. However, the introduction of uncertainty factors requires further analysis, in light of: - the limitations resulting from the application of the traditional NOAEL/NOAEC approach instead of the Benchmark Dose Modelling approach; - the limitations in extrapolating to high-risk subpopulations such as children under one year of age, whose immune system is still immature and who are thus at a very high risk of invasive diseases. https://www.researchgate.net/publication/340268724_Internal_exposure_to_perfluoroalkyl_substances_PFASs_and_biological_markers_in_101_healthy_1-yearold_children_associations_between_levels_of_perfluorooctanoic_acid_PFOA_and_vaccine_response	As also suggested by other stakeholders in the updated opinion a $BMDL_{10}$ was established from the Abraham et al. study as more robust Reference Point. The CONTAM Panel decided not to use additional uncertainty factors, as further explained in replies to Q 151, 171 and 174.
Mamme No Pfas	216	4.4 Risk Characterisation (conclusions)	6521 replace "More longitudinal epidemiological studies are needed on human endpoints, in particular prospective vaccination studies covering more varied types of vaccines, different populations, as well as more studies on other immune outcomes in humans, including risk of infections."	This CONTAM Panel have reviewed these alternative wording suggestions, but consider the original text in the opinion to be appropriate, and therefore any further changes are not considered necessary.

Stakeholder	Comment number	Chapter	Comment	EFSA response
			With More prospective epidemiological studies are needed on human health endpoints, in particular on neonatal effects, testicular and kidney cancer, the response to different vaccinations carried out at different ages, as well as further studies on the risk of infection and other immune outcomes. Populations exposed to high levels of environmental contamination are of primary interest since they provide a large sample of people who have been exposed to prolonged levels of differentiated PFASs. 6526 replace Experimental evidence is needed to understand and quantify the association between PFASs and blood lipids, for example the importance of enterohepatic circulation." With The hypothesis of a confusion between hypercholesterolaemia and concomitant reabsorption of PFASs by the enterohepatic circulation requires further experimental evidence and can be usefully explored in future basic research projects.	
Mamme No Pfas	217	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex J of this Technical report.*	-
Federation of the European Cutlery, flatware, hollowware and cookware industries (FEC)	218	-	*The attachment submitted by this commenter during the public consultation is listed and available in Annex K of this Technical report.*	To answer some of the specific points raised: (i) The CONTAM Panel acknowledges that there is some name confusion for PFAS compounds and Section 1 of the Opinion gives details including full structures and names used within this Opinion. (ii) The CONTAM Panel has noted in the Opinion that short-chain PFASs have shorter half- lives and have differences in toxicokinetics, but that data on these compounds is lacking in terms of both occurrence and data on critical effects.

Stakeholder	Comment number	Chapter	Comment	EFSA response
				(iii) Please note the modifications in the text that have been made to clarify that overall exposure as a result of migration is small compared to other sources, and to clarify the description of the study reported by Choi et al. (2018)

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Abbreviations

BMD	Benchmark dose
BMDL	Benchmark dose lower level
BMDL10	Benchmark dose for a 10% increase
BMR	Benchmark response
bw	Body weight
CES	Critical effect size
COT	Committee on Toxicity
CRP	c-reactive protein
DR	Dose response
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protecion Agency
FCM	Food contact material
FEC	Federation of the European Cutlery
FSANZ	Food Standards Australia New Zealand
HBM	The German Human Biomonitoring
HBGV	Health-based guidance value
HDL	High density lipoprotein
IQR	Interquartile range
LDL	Low density lipoprotein
LB	Lower bound
LOAEC	Lowest-observed-adverse-effect-concentration
LOAEL	Lowest-observed-adverse-effect-level
LOD	Limit of detection
LOQ	Limit of quantification
MoA	Mode of action
MW	Molecular weight
NOAEC	No-observed-adverse-effect-concentration
NOAEL	No-observed-adverse-effect-level
PBPK	Physiologically-based pharmacokinetic (model)
PCBs	Polychlorinated biphenyls
PFASs	Perfluoroalkyl substances
PFBS	Perfluorobutane sulfonic acid
PFCAs	Perfluoroalkyl carboxylic acids
PFDA	Perfluorodecanoic acid

PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOAs	Perfluorooctanoic acid
PFOSs	Perfluorooctane sulfonic acid
PFOSF	Perfluorooctane sulfonyl fluoride
PFPeA	Perfluoropentanoic acid
PFSAs	Perfluoroalkyl sulfonic acids
PFTrDA	Pefluorotridecanoic acid
PFUnDA	Perfluoroundecanoic acid
PND	Post natal day
ppm	Part per million
RPFs	Relative potency factors
SD	Standard deviation
SRBC	Sheep red blood cells
SREBP	Sterol regulatory element-binding protein 1 and 2
TDI	Tolerable daily intake
TK	Toxicokinetic
TSH	Thyroid stimulating hormone
TWI	Tolerable weekly intake
UB	Upper bound
UF	Uncertainty factor
VLDL	Very low-density lipoprotein
WG	Working group
ww	Wet weight

Appendix A – Explanatory note to Public Consultation

EFSA's Panel on Contaminants in the Food Chain (CONTAM) has launched an open consultation on the draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances food. This document presents estimations of human dietary exposure to perfluoroalkyl substances and an assessment of human health risks related to dietary exposure to perfluoroalkyl substances.

Interested parties are invited to submit written comments by 20 April 2020.

Please use the electronic template provided: https://ec.europa.eu/eusurvey/runner/Public_consultation_PFAS to submit comments and refer to the line and page numbers. To submit additional data to support your comments or files, there is an upload function available in the tool (for a maximum size of 1Mb file). Otherwise you can also contact specific unit's functional mailbox: biocontam@efsa.europa.eu

Please note that comments will not be considered if they:

- are submitted after the closing date of the consultation
- are presented in any form other than what is provided for in the instructions and template
- are not related to the contents of the document
- contain complaints against institutions, personal accusations, irrelevant or offensive statements or material
- are related to policy or risk management aspects, which are out of the scope of EFSA's activity.

EFSA will assess all comments which are submitted in line with the criteria above. The comments will be further considered by the relevant EFSA Panel and taken into consideration if found to be relevant. Due to time constraints, EFSA cannot use additional occurrence data submitted during the public consultation for the dietary exposure assessment in this risk assessment. However, occurrence data submitted in SSD format will be stored and used for future risk assessments.

Copyright-cleared contributions

Persons or organizations participating in a Public Consultation of EFSA are responsible for ensuring that they hold all the rights necessary for their submissions and consequent publication by EFSA. Comments should inter alia be copyright cleared by taking into account EFSA's transparency policy and practice to publish all submissions. In case the submission reproduces third-party content in the form of charts, graphs or images, the required prior permissions of the right holder(s) should have been obtained by the PC respondent

Publication of contributions

Contributions will be published (as part of an EFSA report published together with the final opinion) and may be re-used by EFSA in a different context. It should be noted that contributions submitted by individuals in a personal capacity will be published as such, indicating the author's first and family name, unless a substantial justification for protection is provided by the respondent. Contributions submitted on behalf of an

organisation are also made publicly available and attributed to the organization in question.

Submit comments (deadline: 20 April 2020)

Published:
24 February 2020

Annex A – Philippe Grandjean & Esben Budtz-Jørgensen - Upload submission

The following file was submitted by Philippe Grandjean & Esben Budtz-Jørgensen together with their contribution to the Public Consultation:

- **EFSAbiostatComments_EsbenBudtzJ_rgensen.pdf**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4020342

Annex B – German Environment Agency - Upload submission

The following file was submitted by the German Environment Agency together with their contribution to the Public Consultation:

- **Comments_to__EFSA_s_draft_scientific_opinion_German_Environment_Agency_11_1.2**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025273

Annex C – Carlotta Bargossi - Upload submission

The following file was submitted by Carlotta Bargossa as their contribution to the Public Consultation:

- **Contribution_on__PFAS_draft**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025281

Annex D – HBM4EU - Upload submission

The following file was submitted by HBM4EU as their contribution to the Public Consultation:

- **HBM4EU_input_to_EFSA_opinion_on_PFAS__2020.04.17**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025290

Annex E – Rolf Soer - Upload submission

The following file was submitted by Rolf Soer as their contribution to the Public Consultation:

- **Proposal_Total_PFAS_20_April_2020**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025298

Annex F – German Federal Institute for Risk Assessment (BfR) - Upload submission

The following file was submitted by BfR together with their contribution to the Public Consultation:

- **Contribution_BfR_PFAS Attachment**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025304

Annex G – American Chemistry Council - Upload submission

The following file was submitted by the ACC as their contribution to the Public Consultation:

- **ACC_comment_on_PFAS_in_food**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025312

Annex H – National Institute for Public Health and the Environment (RIVM)- Upload submission

The following file was submitted by the RIVM as their contribution to the Public Consultation:

- **BMD_analysis_Abraham_study**
- **BMD_analysis_animal_studies_**

This file was submitted on request from EFSA (in relation to comment number 172):

- **RIVM_PFAS Vaccination Response_Bilinear modelling_29_04_2020**

These files are available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025315

Annex I – The 3M Company - Upload submission

The following file was submitted by The 3M Company together with their contribution to the Public Consultation:

- **2020-04-20_3M_Combined_EFSA_Comments_with_Appendices_A_B_and_C_2**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025326

Annex J – Mamme No PFAS- Upload submission

The following file was submitted by Mamme No PFAS together with their contribution to the Public Consultation:

- **Lettera EFSA**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025330

Annex K – Federation of the European Cutlery, flatware, hollowware and cookware industries (FEC) Upload submission

The following file was submitted by FEC as their contribution to the Public Consultation:

- **Leter to EFSA CONTAM PANEL**

This file is available on the EFSA Knowledge Junction Community on Zenodo at: https://doi.org/10.5281/zenodo.4025332